THE
AMERICAN NATURALIST

VOL. LXXIV *July–August, 1940* No. 753

THE RELATION OF GENETICS TO GEOGRAPHICAL DISTRIBUTION AND SPECIATION; SPECIATION. II.*

SPECIATION IN PEROMYSCUS[1]

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THE summary of the investigations on speciation in the mice of the genus Peromyscus here presented covers studies conducted by myself and my associates over a period of sixteen years. Investigations of Peromyscus were begun much earlier by Dr. Francis B. Sumner, and I have had the advantage not only of the experience of Sumner, but also of the use of his stocks, which he kindly turned over to me at the conclusion of his work with this genus. Much of the field work has been supported in the past by the Carnegie Institution of Washington, through the kindly interest of Dr. John C. Merriam, who until recently was president of the institution. The studies of Peromyscus at the University of Michigan were supported at first through the Museum of Zoology and later through the Laboratory of Vertebrate Genetics. The work at the university has been made possible through

* These three papers conclude the combined symposia given at meetings of the American Association for the Advancement of Science, the first on "The Relation of Genetics to Geographical Distribution and Speciation" at Milwaukee, Wisconsin, before Section F—Zoological Sciences, on June 20, 1939, and the second on "Speciation" at Columbus, Ohio, before a joint session of the American Society of Zoologists and the Genetics Society of America, on December 28, 1939, the first part of which has been published in the May–June, 1940, issue of THE AMERICAN NATURALIST.

[1] Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

the interest and encouragement of President Alexander G. Ruthven.

Evolution within the genus Peromyscus has resulted in the differentiation of forms of varying degrees of distinctness from one another. These various forms may roughly be classified, according to the usage of mammalogists, as local races, subspecies or geographic races, species, species groups, and subgenera.

By local race I mean any local population whose characters differ to a recognizable degree from those of the other local races of the same subspecies. The local race as here defined is, I believe, the same thing as the microgeographic race of Dobzhansky (1937: 146–148). In Peromyscus, and probably in other animals, each more or less isolated intrabreeding local population (gamodeme of Gilmour and Gregor, 1939: 333) actually constitutes a local race, for the studies of Summer (1920: 370–371; 1932: 56–64) and myself have shown that no two local populations of Peromyscus ever have exactly the same characters.

The local races of Peromyscus differ from one another both in the dimensions of the body and skeleton and in pelage color. The differences in certain measurements between the populations of two nearby woodlots may cover nearly the extremes of variation of the particular subspecies concerned (Dice, 1937a: 17–20).

The complexes of characters which distinguish some local races are undoubtedly the result of chance, the combined effect of mutation rate and of the random elimination of genes (Dobzhansky, 1937: 118–148). The characters of other local races are correlated in part with certain features of the environment. In particular, the local races of Peromyscus tend to be relatively pale in coat color on pale-colored soils and dark in color on dark-colored soils (Dice, 1938: 16–18; 1939a: 19–23; 1939b: 13–16). The environment, therefore, through natural selection and possibly in other ways, may play a part in the formation of some local races.

The production of a local race or subspecies of any

kind of animal theoretically requires, as a necessary condition, some degree of isolation from the remainder of the population of the species. This isolation may sometimes, however, be only that produced by distance. The young of most small mammals do not travel very far, on the average, from their place of birth before taking up permanent residences. Accordingly, only a small amount of dispersal takes place in each generation, with the result that there is local inbreeding. That distance alone is an important barrier is demonstrated by the considerable number of subspecies of mammals which are separated from one another only by poorly defined areas of intergradation (Dice and Blossom, 1937: 116–117).

A more important barrier to free interbreeding within a species population than distance alone is the partial isolation caused by the unequal distribution of suitable habitats in nature. The most favorable habitats for a species often occur in patches which are considerable distances apart. Species, therefore, seldom are distributed uniformly over their geographic ranges, but tend instead to occur in more or less isolated colonies. Although there may be frequent interchange of individuals between nearby colonies, a certain amount of inbreeding must occur in each colony. This inbreeding, possibly aided by natural selection and mutation, theoretically will in time lead to the production of distinctive characters in each local population.

The greatest production of local races occurs where barriers are most effective in preventing free interbreeding between the several parts of the whole population of a species. On the deserts of southwestern North America, for example, the local colonies of the cactus-mouse (*Peromyscus eremicus*) on the desert mountains often are separated from one another by many miles of inhospitable desert plain. On these desert mountains many local races have been produced, the pelage color of each of which tends to match the soil color of its local habitat (Dice, 1939a: 19–23).

All the local races of each subspecies of Peromyscus

are, so far as we have been able to test them in the laboratory, fully interfertile. There seem to be no barriers to intercrossing between the several local races of a subspecies other than such physical or ecological barriers as may prevent free dispersal.

The subspecies, or geographic race, according to the usage of the term by vertebrate zoologists, is a larger unit than the local race. It is a division of a species made up of individuals which have certain characters in common and which occupy, usually to the exclusion of the members of other related subspecies, a definite section of the geographic range of the species. That the characters of certain subspecies of Peromyscus are inherited has been demonstrated by Sumner (1924). The subspecies is, however, not necessarily uniform in its hereditary constitution throughout its whole range and some subspecies are probably polyphyletic in origin (Dice, 1940). It is my opinion that the subspecies is best considered to represent an ecologic response, largely of an hereditary nature, expressed by those members of a species which live in a restricted but not always continuous geographic area having a certain type of environment.

The geographic races (subspecies) of Peromyscus have all, so far as they have been tested, proved to be completely interfertile in the laboratory with other geographic races of the same species. In nature, however, two subspecies of the same species may sometimes live in the same district, but fail to interbreed (Dice, 1931). A conspicuous example occurs in Glacier National Park, Montana, where a forest-inhabiting subspecies, *Peromyscus maniculatus artemisiae,* meets a grassland race, *P. m. osgoodi,* with no evidence of interbreeding (Adolph Murie, 1933). The failure of these two subspecies to interbreed in nature is due in part to a difference in their habitats (ecological isolation of Dobzhansky, 1937: 233). The complete separation of the two forms, however, evidently is dependent upon a difference in their mating reactions, for at some places near the margins of their habitats the two races live together without interbreed-

ing. This type of isolation has been called sexual isolation (Gulick, 1905: 84).

The morphological, ecological and psychological differences which separate the subspecies *artemisiae* and *osgoodi* must be assumed to have arisen at a time when the two were not in contact. Subsequently, the ranges of the two forms must have changed so that now the two subspecies meet, without interbreeding, in the Glacier Park region. The two subspecies still are connected together indirectly through chains of intergrading subspecies (Osgood, 1909, pl. 1, where *nebrascensis* = *osgoodi*). Should the chains of intergrading subspecies which now connect *artemisiae* with *osgoodi* ever be broken the two forms would be considered distinct species.

That subspecies are incipient species is believed by many zoologists. To constitute a new form in the process of speciation, however, any part of a species may theoretically be split off, either a group of subspecies, a single subspecies, a part of a subspecies, or a local race. To treat subspecies as incipient species, therefore, is likely to produce the misleading conception that only subspecies can differentiate into species (Dice and Blossom, 1937: 118–120).

An important step in speciation is exhibited by the two species *polionotus* and *maniculatus,* both of which are members of the *Peromyscus maniculatus* species group. These two species differ considerably in body size, but are very similar in other characters. In the laboratory they interbreed fairly well, except that the small *polionotus* females when mated with the larger *maniculatus* males usually die at parturition, due to the large size of the hybrid fetuses. *Maniculatus* females mated to *polionotus* males bear the hybrid offspring without difficulty. Both the female and male hybrids are fully fertile (Watson, unpublished). The close relationship of the two species is proved by their interfertility as well as by their similarity in morphological characters, and they

must accordingly have had a common ancestry. Their geographic ranges, however, are now separated by an area hundreds of miles across where neither occurs. The two forms may be presumed not to have interbred for hundreds of generations, but they have not yet diverged sufficiently in their reproductive processes to have become intersterile.

A somewhat similar relationship is exhibited by the two species *leucopus* and *gossypinus,* both of which are members of the *Peromyscus leucopus* species group. These two species also are closely similar in morphological characters, but *gossypinus* is the larger in body size. In the laboratory they are completely interfertile, and both the female and male hybrids are fertile (Dice, 1937b: 1–3). The ranges of the two species are in general different, but they overlap slightly in the Dismal Swamp region of Virginia and in northern Alabama, and more broadly in the lower Mississippi Valley. Where their ranges overlap they occupy in part the same habitats, but there is no evidence for their interbreeding in nature, except for two presumed hybrids reported from Alabama (Dice, 1940: 18–22). It may be assumed that in the differentiation of these two species they were at one time separated in geographic range, and that during that time they diverged sufficiently in psychology so that in nature they do not now find each other attractive as mates (sexual isolation).

When sterility has developed between two closely related species this sterility of course forms a positive barrier to the interchange of hereditary factors and thenceforth the two species are bound to diverge still farther in evolution. One step in the production of sterility between two species is shown by the relationships between *Peromyscus truei* and *P. nasutus,* both of which are members of the *truei* species group. These two species show only small morphological differences. In the laboratory they cross with some difficulty, and the hybrid males are

all sterile, though the hybrid females are fertile (Dice and Liebe, 1937).

The geographic ranges of the species *truei* and *nasutus* overlap broadly in the arid parts of southwestern North America; there are only slight differences in habitat between them; and the two often occur together in the same ecologic communities. In many places, therefore, no geographic nor ecologic barrier prevents their interbreeding. It is probable, however, that there is a difference in mating behavior (sexual isolation) between the two forms, in addition to their partial sterility, for no hybrids have been found in the wild.

In southwestern North America, where the species *truei* and *nasutus* live, there are many isolated mountains and buttes which carry a belt of juniper and Pinyon pine with habitats suitable for these mice. In many of these isolated situations both species occur; in other places only one is found. There are therefore in this region abundant possibilities for temporary geographic isolation of part of a species with the consequent probability of divergence in evolution. *Truei* and *nasutus* are closely related and must certainly have had a common ancestry. Their differentiation most probably began at some past time when a part of the ancestral species became temporarily separated on some isolated desert mountain.

Complete intersterility between two related species constitutes a more complete separation than partial intersterility, and in most cases is probably the next step in speciation. Many of the species of Peromyscus have reached the stage of complete intersterility. Notably, no two species of Peromyscus, which on the basis of their morphological characters are placed in different species groups or in different subgenera, have ever been successfully hybridized (Dice, 1933: 302–304).

The divergence in hereditary factors between populations isolated from one another should, except for the possible effects of natural selection, theoretically be largely at random. There is, therefore, no reason to

expect that intersterility would be more likely to develop than any other kind of physiological, morphological, or psychological difference. Accordingly, two related but isolated populations might theoretically diverge in morphological characters with little change in psychology or physiology, or they might diverge in psychology or physiology with, for a time, little evident change in morphology. Actually, some Peromyscus races and species which appear very dissimilar are fully fertile together, and conversely, some species which superficially appear nearly alike are completely intersterile.

Ultimately, long-continued isolation will theoretically result in the production of intersterility between the several separated populations, in addition to the production of other differences in physiology, in psychology and in morphology. The evolution of intersterility may, however, by the operation of the laws of chance, be long delayed, and races and species which have become widely different in structure or in behavior may still be potentially interfertile.

That sexual isolation is one of the most important factors in the speciation of Peromyscus is evident from these studies. Numerous subspecies and species which are fully fertile together under laboratory conditions fail to interbreed in nature, even when they at times occupy the same habitats. If a difference of some sort in the mating psychology of these forms did not exist, they would almost certainly interbreed in nature and thus merge their identities. It is evident, moreover, that once sexual isolation has become established between two forms of common ancestry their divergence is likely to continue until intersterility ensues and separates them irrevocably.

Although geographic isolation is, I believe, an essential first step in the splitting of a species, it is evident that, at least in Peromyscus, geographical isolation often does not continue long enough for intersterility to arise. Inherited modifications in behavior leading to sexual iso-

lation between two parts of a species seem more quickly developed. After sexual isolation has arisen, separating two parts of a species, those parts will thenceforth remain distinct, even though their geographic isolation is later broken down. In time, under the protection of this sexual isolation, intersterility between the two daughter forms will be expected to develop, completing their specific differentiation.

Ecological isolation, produced by a divergence in psychology between two parts of a species so that each selects a different sort of habitat, is of some importance in separating forms which otherwise would likely interbreed. However, ecological isolation, when acting alone, seems unlikely ever to be effective completely. In nature there nearly always are some intermediate conditions between the several habitat types, and in these intermediate situations ecological isolation is likely to break down. Ecological isolation, however, undoubtedly has considerable effectiveness in speciation at those places where it is associated with and reinforces sexual isolation.

Summary

The forms differentiated within the genus Peromyscus may roughly be classified according to degree of distinction as local races, subspecies or geographic races, species, species groups, and subgenera. Different subgenera and different species groups, so far as they have been tested in this genus, are completely intersterile. Within the *truei* species group two included species are partially intersterile. In two other Peromyscus species groups the included species are potentially interfertile, but in nature are separated partly by geographical and partly by psychological (sexual) barriers, so that no interbreeding occurs. Within any given species of Peromyscus all the subspecies, so far as they have been tested, are potentially interfertile, but in nature some adjacent subspecies are separated by geographical, by ecological or by sexual barriers. Sexual isolation

seems in this genus to be of especial importance in speciation.

LITERATURE CITED

Dice, Lee R.
1931. *Jour. Mammalogy*, 12: 210–213.
1933. *Jour. Mammalogy*, 14: 298–305.
1937a. *Occ. Papers Mus. Zool. Univ. Mich.*, 352: 1–32, 1 map, 2 figs.
1937b. *Contrib. Lab. Vert. Gen. Univ. Mich.*, 4: 1–3.
1938. *Occ. Papers Mus. Zool. Univ. Mich.*, 375: 1–19, 1 map.
1939a. *Contrib. Lab. Vert. Gen. Univ. Mich.*, 8: 1–27, 1 map.
1939b. *Contrib. Lab. Vert. Gen. Univ. Mich.*, 12: 1–22, 1 map.
1940. "Ecologic and Genetic Variability within Species of Peromyscus." AM. NAT. In press.
1940. *Jour. Mammalogy*, 21: 14–23, 1 map.
Dice, Lee R., and Philip M. Blossom
1937. Carnegie Inst. Wash., Publ. 485. iv + 129 pp., 8 pls., 8 figs.
Dice, Lee R., and Margaret Liebe
1937. *Contrib. Lab. Vert. Gen. Univ. Mich.*, 5: 1–4.
Dobzhansky, Th.
1937. "Genetics and the Origin of Species." New York: Columbia Univ. Press. xvi + 364 pp., 22 figs.
Gilmour, J. S. L., and J. W. Gregor
1939. *Nature*, 144: 333.
Gulick, John T.
1905. Carnegie Inst. Wash., Publ. 25. xii + 269 pp., 2 maps, 3 col. pls.
Murie, Adolph
1933. *Univ. Mich. Occ. Pap. Mus. Zool.*, 270: 1–17, 2 figs.
Osgood, Wilfred H.
1909. *N. Amer. Fauna* (Bur. Biol. Surv.), 28: 1–285, 8 pls., 12 figs.
Sumner, F. B.
1920. *Jour. Exp. Zool.*, 30: 369–402, 7 figs.
1924. AM. NAT., 58: 481–505.
1932. *Biblio. Genetica*, 9: 1–106, 24 figs.

LEVELS OF DIVERGENCE IN DROSOPHILA SPECIATION[1]

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To evaluate the stage of divergence occupied by two organisms in terms of the evolutionary roads over which they have traveled from a common origin and their present place in the space-time continuum of nature is a difficult task. With the knowledge at hand to attempt this for the many complexly interrelated forms in any large group such as that delimited by our present definition of the genus Drosophila is to undertake the impossible. However, the venture to summarize some of the relevant data may serve to formulate more clearly the problems involved.

There is, indeed, one fact which favors analysis. This is the rather thoroughly demonstrated principle that at its primary level evolution is built out of discrete and discontinuous blocks, the occurrence of mutations, using this term in the broadest sense, and their distribution among individuals making up populations finite in number. The actual analysis of the evolutionary status of any group of organisms at a given time-level will depend upon a fair sampling of the genetic structure of the populations which make up this group.

For any subdivision of the genus Drosophila, other than such isolated populations as occupy half-pint milk bottles the world over, the number of individuals is so large, the distribution of populations so extensive, and the fluctuations in their structure so frequent, that a rough approximation of genetic analysis is all that can be expected. With this understanding of the limitations of analysis, let us examine some of the data.

[1] Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

DIVERGENCE AT THE GENE MUTATION LEVEL

We shall assume that the primary level of evolution and speciation has to do with the occurrence and frequency distributions of mutations, genic or chromosomal, in populations made up of individuals which are capable of interbreeding freely when the opportunity is offered. It should be understood that the determination of the frequency of gene and chromosomal mutations of the types easily observed establishes a pattern of population structure which may readily be compared with that for mutations not well adapted to ready laboratory analysis, but which may play a more significant evolutionary role than their morphologically more conspicuous fellows.

Over ten years ago Tschetverikov (1927) and the Timoféëffs-Ressovsky (1927) demonstrated by collecting and inbreeding samples of *Drosophila melanogaster* populations that mutant genes were present in heterozygous condition in large numbers. To those who were wont to claim that in some way the laboratory housing and feeding of these animals was conducive to stimulating a sort of germinal upset, these findings must have come as something of a shock.

The most extensive work on the analysis of *Drosophila melanogaster* populations has been done by Dubinin and his colleagues (1934). Wild flies were collected from ten localities in Russia. By the use of special methods, 3,766 autosomes were carefully tested for the presence of lethal and visible mutations. From one locality 84 chromosomes yielded 18 lethals and 26 visibles; from another, 184 chromosomes gave no visibles. Recently Ives (1939) has reported on 151 second chromosomes from a South Amherst population of the same species. He recovered 47 lethal-bearing chromosomes, 18 semi-lethal chromosomes and 12 of the non-lethal bearing chromosomes which carried visibles. Thus Ives found that over 50 per cent. of the chromosomes tested carried mutants.

These data show great differences in the percentage of mutations carried in various populations of *melano-*

gaster. In the investigations by the Timoféëffs in Germany, Tschetverikov and Dubinin and others in Russia, Ives in the United States and Gordon (1936) in England, the *melanogaster* populations studied were breeding outside the natural range of the species. *Melanogaster* is tropical and can not overwinter in the open in any of these geographical areas. The populations are cut down to relatively few individuals overwintering indoors, then attain a peak in late summer and autumn with abundant food and proper humidity and temperature. Year after year small samples of the autumn population fortuitously survive and expand into the next year's maximum. This mechanism provides that in a species with many mutating loci certain local populations will show high frequencies of one or a small group of mutant genes; other populations will show few mutant genes and samples of considerable size may prove to be entirely mutant free. This latter situation seems to have been met with in one Russian population, which may be supposed to have suddenly "bred up" from a small local focus.

Similar studies on lethal gene distribution in populations of *Drosophila pseudoobscura* from the natural breeding range have been made by Sturtevant (1937), and on both lethals and visible mutants for the same species by Dobzhansky and Queal (1938b). In general, the findings are consistent with the view that on the average the effective or minimum breeding population is not cut so low at critical periods in wild populations of *pseudoobscura* in its natural range as in the semi-wild populations of *melanogaster* outside the natural range.

Divergence at the Chromosome Inversion Level

Of the several ventures into the field of Drosophila population analysis, the most extensive in scope and significant in results is the undertaking of Dobzhansky and Sturtevant (1938) to sample populations of *Drosophila pseudoobscura,* races A and B, from many localities throughout their geographical range. The salivary

chromosome analysis of the third chromosome has led to their construction of the probable pattern of relationship between seventeen gene sequences in this chromosome. Through the reasonable assumption that two gene sequences which may be derived one from the other by a single inversion are more closely related than two which differ by more than one inversion, it was possible to trace step by step the evolution of these chromosome sequences. Perhaps the strongest evidence for the validity of the findings was the postulation during the course of the investigation of certain hypothetical alignments which were later actually discovered. The authors of this work do not claim that it gives a complete picture. Certainly it sets a standard for the study of chromosome evolution in animals favored with salivary chromosomes which undergo inversion on an extensive scale.

Recent studies of a similar nature have been reported by Dobzhansky and Socolov (1939) on *Drosophila azteca*, and by Miller (1939) on *Drosophila algonquin*.

Dobzhansky and Queal (1938a, 1938b), Koller (1939) and Dobzhansky (1939a, 1939b) have reported on the proportions of gene rearrangements and lethal mutations in *Drosophila pseudoobscura* occupying separate mountain ranges and canyons in one range near Death Valley, and certain microgeographical areas in Texas and in populations from the highlands of Mexico and Guatemala. The data secured are consistent with the hypothesis that effective breeding populations of small size tend to eliminate some genetic variability and to give rise to high frequency of certain types. Larger effective breeding populations contain more genetic variability with less concentration of specific variants. The findings are in line with the conclusions of Wright (1931) based on mathematical analysis. As a result of these eco-genetic studies we may conclude that local populations, fluctuating year after year and in shorter time intervals in response to conditions of the environment, present the possibility of the fixation and spread of new types here and there over the range of the species.

Divergence at the Level of Inter-fertile Races or Subspecies

Whatever their method of origin, it is of interest to examine the genus for cases of the fixation of an ensemble of hereditary variations. Sturtevant and Dobzhansky (1936) have described *Drosophila affinis iroquois,* differing from typical *affinis* in darker body and leg color. The locality records indicate that *affinis* tends to be distributed through southeastern United States and to be replaced northwards by *iroquois. Mahican* was described at the same time as a subspecies of *athabasca,* somewhat lighter in color and with a distribution in the eastern United States as contrasted to the western range of *athabasca.* Subspecific crosses are in both cases fully fertile and the differences between these forms are so slight as to make determination difficult. These cases represent fixation at a primary level of morphological divergence, and the forms appear to differ less from each other than many types which might be extracted from the genetic variability of the average Drosophila population.

In 1938 Sturtevant sent to our laboratory stocks of *hydei*-like flies collected in Yucatan, Mexico, by M. Steggerda and called attention to the light thoracic pattern. We have described them (in press) as a new subspecies, *Drosophila hydei yucatanensis,* differing from typical *hydei* in a reduction in size of thoracic pigment spots, larger eyes, a squattier body build and other minor characters. This form breeds true, crosses readily with *hydei,* the hybrids are perfectly fertile and the hybrid salivaries appear to contain no major aberrations. Morphologically, the hybrids are intermediate, but the characters are such that they could be studied only by statistical methods under careful environmental control.

This past summer Harrison Stalker and I trapped two males and two females of a fly near Overton, Ohio. We shall describe it as a new subspecies of *Drosophila macrospina. Drosophila macrospina ohioensis,* subspecies

nova, differs from typical *macrospina* (see Stalker and Spencer, 1939) in the following characters. It has a darker eye in contrast to the bright red eye of *macrospina*. The fleck or shadow in the eye of *ohioensis* is less distinct and the eye is smaller than that of *macrospina*. *Ohioensis* has a lighter brown thorax and abdomen, is a little larger on the average and has a less squatty body build. Type *macrospina* was collected in Austin, Texas, in 1935, and sent to us through the courtesy of Dr. Patterson. *Ohioensis* has been taken from near Overton and from a point six miles north. These two subspecies cross readily and the hybrid offspring are quite fertile. Preliminary examination of the hybrid salivaries indicates relatively minor aberrations. Hybrid characters are intermediate to the parent forms. Of the four cases mentioned, this last one probably represents the greatest divergence; however, it would appear that if the two forms of *macrospina* occupied the same habitat range this divergence would rapidly be swamped by intercrossing.

DIVERGENCE AT THE LEVEL OF PARTIAL INTER-SUBSPECIES AND HYBRID STERILITY

Accepting the criterion of inter-group and F_1 hybrid sterility as the soundest basis for seriating different levels of speciation, we may next consider cases in which lowered fertility occurs in inter-group crosses and their hybrids.

Drosophila virilis americana (Spencer, 1937) differs from *virilis virilis* in many morphological and physiological characters, including eye size and color, body- and pupa-case colors, etherization time and pupation habits. When these forms are crossed a few hybrid offspring are produced. The inter-fertility varies greatly with the stock of *virilis* used. Hybrids of both sexes are partially fertile when crossed *inter se* or back to either parent subspecies. In fact, on the average, hybrid fertility is considerably higher than that of the initial cross. Hughes

(1939) has recently published a full account of the cytology of this case. In the metaphase of the ganglion cells of *virilis* there are five pairs of rod-shaped chromosomes and one pair of dots in both sexes. In *americana* the female has two pairs of V-shaped chromosomes, a pair of rods and a pair of dots. In the male there is one pair of V's, one pair of rods, a pair of dots and a single V paired with two rods. The rod-like X chromosome of *virilis* has its homologue in one limb of an *americana* V. The other limb corresponds to a *virilis* autosome. The Y of *americana* is not fused with an autosome; hence the peculiar configuration in the male of *americana*. The salivary chromosomes of both subspecies show five long units and one very short one. In the hybrids the units corresponding to the X, second, fourth, and fifth chromosomes show configurations which indicate that these chromosomes differ by inversions in the two subspecies. Hybrid chromosome five shows very loose pairing.

With cross-sterility as the criterion for determining species, the *virilis* case presents a taxonomic problem. This is augmented by the great difference in cross-fertility between *americana* and the several *virilis* strains tested. In this connection some extremely interesting new material on a group of partially inter-fertile forms in the *virilis* complex is being presented in the genetics demonstrations at these meetings by Patterson and his colleagues (1939).

Recently a new case of hybridization between forms somewhat more distinct than *virilis* and *americana* and producing partially fertile hybrids has been discovered. Last July 27th Stalker and the author collected two new Ohio Drosophila in the Killbuck Swamps, Wayne County. One of these has been identified by Sturtevant as *Drosophila lattivittata* Malloch. However, this name is invalid through its prior use for another species. The other form is undescribed. We shall name and describe these forms elsewhere. However, the case is pertinent to

the present discussion and we therefore present a preliminary account.

Upon subsequent collecting, over eighty specimens of the two forms were taken. *Lattivittata* was the more abundant. Both have a beautiful striped and spotted color pattern, and appear to be related to the quinaria-transversa complex. The two differ in many morphological characters including size of wing clouds, shape and color of the eye, body color, shape of posterior cross-vein, length and color of pupal horns and external genitalia. Stalker states from tests made in August and September that "both reciprocal crosses are made if large numbers of individuals are used. The hybrid salivaries present a tangled picture, with long regions of loosely paired chromosomes."

The hybrids of *lattivittata* females by males of the other species have eyes intermediate but more like their mothers in shape, and wing cloud and cross-vein shape more like that of the fathers. In the reciprocal cross all these characters more closely resemble those of the mothers. This indicates that eye shape is conditioned either by maternal effects or by sex-linked genes. The hybrids of the cross made in either direction proved partially fertile in mass cultures, though fertility was observably lower than for either parent species. These two forms will probably be described as new species, though they seem to occupy a position as regards hybrid sterility not far removed from the *virilis-americana* complex. They occupy apparently a sharply defined ecological habitat. We have collected many thousands of Drosophila in woodland and other areas in Wayne County without taking a single specimen of either species. The discontinuous nature of swamp habitats should offer peculiar advantages for the isolation and development of new variant forms. The problems of ecology so important to an understanding of the factors involved in speciation should be more readily solved in forms known to occupy a definitely delimited habitat. We have al-

ready collected the larvae and pupae of one of these species breeding on the decaying leaf stalks of the broad-leaved arrowhead, *Sagittaria latifolia.*

In the process of speciation both the mechanism and relative time and course of development of incompatibility of the two diverging forms and of the sterility of their F_1 hybrids may quite conceivably differ. Put in its simplest form the question resolves itself into whether F_1 sterility develops prior or subsequent to the inability of the parental forms to produce viable offspring. A third alternative suggested by Sturtevant (1938) is that of initial partial sterility of the hybrids leading to selection of factors favoring the elimination of inter-group crossing. We find in the *virilis* and *lattivittata* cases evidence for the development of a strong incompatibility of the parent forms prior to the development of equally marked hybrid sterility. In the *virilis* case the incompatibility seems to depend largely on psychological factors, less frequent cross-matings and/or less sperm transferred to the female at a mating. We do not suggest that this is a general rule and strongly suspect that the mechanism involved varies from case to case.

Divergence at the Level of Partial Inter-group and Complete Hybrid Sterility in One Sex

The *Drosophila pseudoobscura,* races A and B, and *Drosophila miranda* complex illustrates the stage at which complete hybrid sterility has been attained in one sex, in this case the male. We find it impossible to review adequately in the space allotted the thorough analysis of this material due largely to the researches of Dobzhansky.

In brief, races A and B of *pseudoobscura* were first recognized by Lancefield (1929) as producing sterile male hybrids. The two races are morphologically identical by the usual criteria. Mather and Dobzhansky (1939) have demonstrated slight size differences by statistical methods. Race B, broadly speaking, is northern in its distribution from British Columbia to California, while

race A has a wider range including most of the race B territory and extending eastward into Nebraska and Texas and south into Mexico and Central America. Dobzhansky (1937a) states, "It is solely because of the lack of externally visible distinctions that these two forms are described as races of the same species. By any other criterion they should be considered distinct species." Boche (Dobzhansky, 1937b) has demonstrated partial sexual isolation between race A and race B. Inter-racial crosses occur less readily than intra-racial crosses. Male hybrids in both reciprocal crosses are entirely sterile; female hybrids are partially fertile when crossed to either parent race, and these crosses give rise to a series of types differing in fertility due to various combinations of the parental chromosome complexes. Hybrids show inversions in the salivaries (Tan, 1935), though in view of the prevalence of inversions within races the inter-racial differences are not extreme. Dobzhansky (1937b) has found that the sterility of hybrid males is due to genetic factors carried in all four of the long chromosomes.

Drosophila miranda, first collected by Boche and described by Dobzhansky (1935) is larger than *pseudoobscura,* though morphologically quite similar. This species will cross with either race of *pseudoobscura.* The females from crosses in either direction are normal in appearance and MacKnight (1939) reports that some of them are slightly fertile. The males are completely sterile and differ in both form and viability, depending on the direction of the cross. The chromosome mechanism of *miranda* includes two pairs of X's in the female and a Y and two single X's in the male. Dobzhansky and Tan (1936) have shown through study of the hybrid salivaries of *pseudoobscura* by *miranda* that there are more differences in the arrangement of the chromatin than in any two hybridizing forms thus far mentioned. Most of these involve inversions within chromosome arms.

Divergence at the Level of Complete Hybrid Sterility

The next step in evolutionary divergence may be represented by the two species, *Drosophila melanogaster* and *Drosophila simulans.* Sturtevant (1919) first described *Drosophila simulans,* which has larger eyes, narrower cheek, darker body color and stouter body build than *melanogaster.* The egg filaments of *simulans* are longer, and the male genitalia differ more than in any other hybridizing forms of Drosophila known. The cross of *melanogaster* female by *simulans* male is more often successful than the reciprocal. Hybrids of both sexes are entirely sterile. Horton's (1939) study of the hybrid salivaries shows the presence of ten chromosomal rearrangements, one long and four very short inversions, and four minor changes in banding at ends of chromosomes. Natural intra-specific chromosome rearrangements in *melanogaster* are rare. This may account for the minor changes in this case as contrasted to some others.

In the *affinis* group Bauer and Dobzhansky (1936) have reported on crosses of *Drosophila azteca* females to *Drosophila athabasca* males. Hybrids of both sexes are entirely sterile and the hybrid salivaries show marked differences in chromosome alignments. Miller (1939) has secured sterile hybrids from the cross of *algonquin* by *athabasca.*

In addition to the *virilis* complex, I understand that Patterson and his colleagues at the University of Texas are working on other cases involving hybridization in Drosophila.

In conclusion, we have attempted to review some of the pertinent data dealing with levels of divergence in Drosophila speciation. Starting with variations at the single mutation, genic or chromosomal, stage we have presented cases seriated in terms of inter-crossing forms and increasing hybrid sterility. A survey of these data presents many interesting problems. One of these is the variation in the pattern of evolution from group to

group. In some cases outward morphological changes have remained at a minimum, while major changes in gene realignments and sterility have taken place. In other cases fertility has remained close to normal, but morphological changes in geographically isolated groups have occurred.

Clearly emerges the concept of a vast array of micro- and macro-populations of different Drosophila groups, fortuitously fluctuating in size and distribution in time and space in response to changing ecological factors. Academic discussions of natural selection on populations indefinitely large and in breeding equilibrium are obsolete as far as the genus Drosophila is concerned. Equally obsolete are purely historical discussions of evolution when the process is taking place all about us.

Finally, with the mass of accumulated cyto-genetic data on *Drosophila melanogaster,* the incomparable tool of salivary chromosome analysis and the ease and speed of culture, Drosophila offers unique opportunities for further ventures into the old but always alluring field of the "Origin of Species."

LITERATURE CITED

Bauer, H., and Th. Dobzhansky
1936. *Rec. Genet. Soc. Amer.,* 5: 185.
Dobzhansky, Th.
1935. *Genetics,* 20: 377–391.
1937a. AM. NAT., 71: 404–420.
1937b. "Genetics and the Origin of Species." New York: Columbia University Press.
1939a. *Genetics,* 24: 391–412.
1939b. *Proc. Nat. Acad. Sci.,* 25: 311–314.
Dobzhansky, Th., and M. L. Queal
1938a. *Genetics,* 23: 239–251.
1938b. *Genetics,* 23: 463–484.
Dobzhansky, Th., and D. Socolov
1939. *Jour. Hered.,* 30: 3–19.
Dobzhansky, Th., and A. H. Sturtevant
1938. *Genetics,* 23: 28–64.
Dobzhansky, Th., and C. C. Tan
1936. *Z. indukt. Abstamm. u. VererbLehre,* 72: 88–114.
Dubinin, N. P., and collaborators
1934. *Biol. Zh. (Mosc.),* 3: 166–206.

Gordon, C.
1936. *Jour. Genet.*, 33: 25–60.
Horton, Ira H.
1939. *Genetics*, 24: 234–243.
Hughes, R. D.
1939. *Genetics*, 24: 811–834.
Ives, Philip T.
1939. "A High Frequency of Lethal Mutations in a Wild Population of Drosophila." Abstract presented at 7th Int. Cong. Gen.
Koller, P. C.
1939. *Genetics*, 24: 22–33.
Lancefield, D. E.
1929. *Z. indukt. Abstamm. u. VererbLehre*, 52: 287–317.
MacKnight, R. H.
1939. *Genetics*, 24: 180–201.
Mather, K., and Th. Dobzhansky
1939. Am. Nat., 73: 5–25.
Miller, Dwight D.
1939. *Genetics*, 24: 699–708.
Patterson, J. T., Wilson Stone, and A. B. Griffen
1939. *Rec. Genet. Soc. Amer.*, 8: 131.
Spencer, Warren P.
1937. *Rec. Genet. Soc. Amer.*, 6: 169.
"Subspecies, Hybrids and Speciation in *Drosophila hydei* and *Drosophila virilis*." In press.
Stalker, Harrison D., and Warren P. Spencer
1939. *Annals Ent. Soc. of Amer.*, 32: 105–112.
Sturtevant, A. H.
1919. *Psyche*, 26: 153–155.
1937. *Biol. Bull.*, 73: 542–551.
1938. *Quart. Rev. Biol.*, 13: 333–335.
Sturtevant, A. H., and Th. Dobzhansky
1936. Am. Nat., 70: 574–584.
Tan, C. C.
1935. *Genetics*, 20: 392–402.
Timoféëff-Ressovsky, H. A., and N. W. Timoféëff-Ressovsky
1927. *Arch. EntwMech. Org.*, 109: 70–109.
Tschetverikov, B. S.
1927. *Verh. V. int. Kongr. Vererbungsw.*, 1499–1500. *Z. indukt. Abstamm. u. VererbLehre*, Suppl. II, 1928.
Wright, S.
1931. *Genetics*, 16: 97–159.

SPECIATION AS A STAGE IN EVOLUTIONARY DIVERGENCE[1]

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Two principal components may be recognized in the process of evolution. First, during the course of evolution the diversity of organisms is increased. Second, evolution involves the development of discontinuity in the living world, since the organisms become segregated into discrete arrays termed races, species, genera, etc. The increase in diversity is accomplished through the production of new genetic variants, mutations in the broad sense of this word, and is counteracted by heredity which tends to preserve the similarity between succeeding generations. The discontinuity is produced by isolation which hampers or prevents the interbreeding of the members of different discrete arrays. On the race level, the geographical isolation is most important; on the species and higher levels, various physiological isolating mechanisms become increasingly more and more effective. Isolation has its antithesis in the mechanism of sexual reproduction and Mendelian recombination which tends to reunite the discrete groups into a single variable mass.

By "speciation" one may understand two different things. One may apply this term solely to the process of the formation of the discrete non-interbreeding arrays, or one may apply it to include the production of variability as well. In the latter case, the term "speciation" becomes synonymous with "evolution." This is more than a question of words; a real problem is here involved which has not been heretofore very clearly stated, much less solved. In a nutshell the problem is whether isolating mechanisms develop as a necessary consequence of the

[1] Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

accumulation of genetic differences in general, or whether they represent a separate category of genetic changes which appear and become established only under certain special conditions. In discussing this problem two fundamental considerations must be kept in mind. First, the genetic information available shows that the differences between natural races, species, and genera are complex, consisting of numerous genic as well as chromosomal elements. Second, the maintenance of species as discrete entities is possible provided only that their interbreeding be prevented by isolating mechanisms. Unless two or more species are isolated geographically, they must either develop physiological isolation or become fused into a single greatly variable species. In the process of species divergence a complex of genic and chromosomal differences must be formed, and one or more physiological isolating mechanisms must become established.

The traditional view-point, which is implied, if not expressly stated by most taxonomists and geneticists, is that species formation is a graduated and uniform process. The usual view is that if groups of individuals are confined to different territories, and if sufficient time be allowed during which these groups remain separated, they will become distinct first as races, then as species and, finally, as genera or higher categories. This seems to mean that the development of isolating barriers is a necessary corollary to the accumulation of genetic differences. Unless such a simple relation between the genetic differences at large and the appearance of physiological isolation can be demonstrated, the above view is invalid. The alternative seems to be that the development of physiological isolation is a process separate from that which produces the gene complexes responsible for other morphological and physiological differences between the incipient species.[2]

[2] Apart from the above alternatives stands the unorthodox opinion of Goldschmidt (1932, 1933) who sees no bridge between raciation and speciation. To be sure, Goldschmidt nowhere states that the essence of species differentiation lies in the presence of physiological isolating mechanisms,

The only method of speciation which is at all clearly understood is that by polyploidy. A new species may emerge abruptly following a reduplication of the chromosome complement. It is known in a number of instances that polyploids are crossable only with difficulty to the parental species from which they have been derived, although they are capable of sexual reproduction with their like (Karpechenko, 1928 and others). Hence, in these cases, a physiological barrier arises simultaneously with the formation of a new species. However, species formation by polyploidy is confined to certain groups of organisms, mostly plants. The more general method of speciation is through a gradual accumulation of genic and chromosomal changes. Here the problem of the origin of isolating mechanisms is by no means settled.

It would appear, however, that the accumulation of genetic changes does not necessarily induce isolation. For example, in Drosophila strains may be synthesized which differ in more than a dozen genes, but there is not the slightest evidence that the crossability of such strains is lowered. This is not a conclusive proof that strains differing in hundreds of genes would show no limitation of interbreeding, but from all that we know at present this seems very doubtful. So much for genic changes. Structural changes in chromosomes, especially translocations, do produce partial sterility in heterozygotes. Yet, wild species are known which show less alteration of the chromosome structure than can be produced in the laboratory without at the same time inducing isolation. Thus, the so-called "races" of *Drosophila pseudoobscura* (which actually behave as distinct species) produce semisterile hybrids when crossed, but differ at most in four inverted sections in their chromosomes. In *D. melanogaster,* heterozygotes for five inversions are fertile, and in *D. pseudoobscura* itself heterozygotes for quintuple in-

but this is the only interpretation that the present writer is able to conceive of his views. Otherwise, the evidence for continuity between races and species is overwhelming.

versions in the third chromosome occur in nature without showing a lowering of the reproductive potential (Dobzhansky, 1937). Although in *D. melanogaster* strains have been constructed artificially which produce no viable offspring when crossed to the original form (Stern, 1936; Kozhevnikov, 1936), the differences which exist between wild species have not been copied.

The problem may be approached from another angle, namely, by studying the genetic basis of the isolating mechanisms actually encountered in nature. Such studies might reveal whether a relation exists between the genetic factors producing isolation and the factors causing morphological and physiological differences between species. The sterility of hybrids between the "races" of *Drosophila pseudoobscura* proved to be due to numerous complementary genes scattered apparently in all the chromosomes (Dobzhansky, 1937b and other publications). Furthermore, within each race genetic variants were encountered which were similar to, or identical with, the genes composing the sterility barrier between the races. Thus, one can visualize the building up from the genetic elements available within a species of the sterility barrier which separates species. The point which interests us most at present remains, however, not settled: It is unknown whether the "sterility genes" produce, by manifold effects, the morphological and physiological differences between the races, or whether separate groups of "morphological" and "physiological" genes on the one hand and of "sterility" genes on the other are involved. The latter alternative seems, however, more probable.

As indicated above, the view which seems most likely to be correct on the basis of the genetic information available at present is that the origin of isolation is a process separate from that of the origin of other species differences. An attempt can now be made to outline a theory that would help to visualize the interrelations between these two processes. This theory starts with the

premise that each species, genus and probably each geographical race is an adaptive complex which fits into an ecological niche somewhat distinct from those occupied by other species, genera and races. The adaptive value of such a complex is determined not by a single or a few genes, but is a property of the genotype as a whole. Furthermore, the adaptive complex is attuned to its environment only so long as its historically evolved pattern remains, within limits, intact. It is true that interbreeding of different adaptive complexes may sometimes result in emergence of new genotypes which fit into unoccupied or sparsely settled ecological niches—hence the evolutionary role of hybridization. Nevertheless, hybridization usually leads to the formation of disharmonious recombinations.

Considerations such as these have prompted some writers (Dobzhansky, 1937a, b; Sturtevant, 1938; *cf.* Fisher, 1930) to assume that occurrence of hybridization between races and species constitutes a challenge to which they may respond by developing or strengthening isolating mechanisms that would make hybridization difficult or impossible. Where hybridization jeopardizes the integrity of two or more adaptive complexes, genetic factors which would decrease the frequency or prevent the interbreeding would thereby acquire a positive selective value, even though these factors by themselves might be neutral. Race formation is essentially the development of genetic patterns which are adapted to a definite environment. Speciation is a process resulting in fixation of these patterns through the development of physiological isolating mechanisms. Clearly, raciation and speciation should not be conceived of as entirely independent processes, but the development of physiological isolating mechanisms must nevertheless be supposed to intervene only after the divergence of the adaptive complexes had been initiated. If races are to become species, isolating mechanisms must arise when the distinct adaptive complexes are exposed to the risk of disintegration due to

interbreeding. It may be of interest to discuss some evidence that appears to support or raise obstacles to this theory.

Races of the same species are, as a rule, confined to different territories. Not infrequently races merge into one another; in passing from the territory of one race to that of another, geographical gradients, or to use the term recently proposed by Huxley (1939), "clines," in the differentiating characters are encountered. The clines are seldom perfectly gradual; more commonly the cline is much steeper in a certain zone than in others. The zone of the steep cline is the geographical boundary between the races. It is in these zones that the exchange of the elements of the gene complexes of the races is most frequent, and hence the formation of isolating mechanisms of any kind is most easily accomplished. The traditional belief seems to be that a race first becomes a species at the center of its distribution; the view here advanced implies that speciation is initiated primarily at the boundaries between races.

The lowering of the adaptive value of the recombination products from interspecific crosses is attested both by experimental data and by observations in nature. An excellent case of this kind has been described by Meise (1928). The distribution areas of the two crows, *Corvus corone* and *Corvus cornix,* are apposed to each other along a line some 3,000 kilometers long winding across Europe and Asia. A narrow zone on either side of this line is populated by obvious hybrids, and yet the interbreeding products do not seem to diffuse toward the main bodies of the parental species. Meise presents convincing evidence to show that *C. corone* and *C. cornix* were separated during the Ice Age, and that their distributions converged in the post-Glacial time. Nevertheless, the hybridization zone seems to be even narrower where the contact between the species had been established long ago than it is where the contact is more recent. This is an example of a situation where physio-

logical isolation has not yet developed despite the occurrence of interbreeding, and despite the obvious inferiority of the recombination products. It can hardly be doubted that any genes which prevent or diminish the frequency of hybridization would have a positive selective value, at least in the boundary zone. We are confronted here with that critical stage of speciation where the classification of two forms as races or as species is arbitrary.

An interesting regularity has been revealed by the study of the geographical distribution of the factors that modify the extent of the sexual isolation between *Drosophila pseudoobscura* and *Drosophila miranda*. The area of *D. miranda* is broken into two parts, one in the Puget Sound region and the other in the central Sierra Nevada. Both parts are included in the distribution of *D. pseudoobscura* which extends from British Columbia to Guatemala. As a rule the strains of *D. pseudoobscura* from the Puget Sound region show the strongest, and those from places remote from Puget Sound, an intermediate or weak isolation from *D. miranda*. Moreover, the genetic factors which modify the sexual isolation between *D. pseudoobscura* and the northern race of *D. miranda* are distinct from those which influence the behavior of *D. pseudoobscura vis-à-vis* the southern race of *D. miranda*. It is evident that the genes increasing the isolation between *D. pseudoobscura* and the Puget Sound *D. miranda* are of highest selective value in those populations of the former species which reside in or near the area where the latter species also occurs (Dobzhansky and Koller, 1938).

That isolating mechanisms may develop in the regions where the interbreeding of related species is taking place is plausible enough. One of the difficulties with the theory lies in explaining how the gene complexes responsible for the isolating mechanisms come finally to permeate the whole bodies of these species. What selective value have the isolating mechanisms for those parts of the species which are not, and never were, exposed to the danger of interbreeding with other species? A possible

answer to this question is that if certain genes are favorable in a part of the species area and neutral elsewhere, they will eventually diffuse throughout the species by migration. The diffusion will certainly require time, and hence one may expect the degree of isolation between two species to depend upon the geographic origin of their representatives. As stated above, this is the case in *Drosophila pseudoobscura* and *D. miranda.* Here is an almost totally unexplored field for future studies. It must also be taken into account that "isolating genes" must be considered as an integral part of the species genotype as a whole. The introduction of new elements in the form of the originally neutral "isolating genes" may eventually cause a general reconstruction of the species genotype so that these genes may acquire the role of an essential part of the system, and hence an adaptive value outside of their original province of begetting isolation.

Another difficulty is with species isolated on oceanic islands or in similar situations. If the geographical barriers between races are secure enough, the precondition for the development of physiological isolation is absent. Yet, insular speciation is a classic example of speciation in general. Precisely how serious is this difficulty is not clear, since experimental data on the presence or absence of physiological isolation between insular species seem to be lacking; this is another fertile field for future work. Cases are known in which a species having developed on an island subsequently migrates to the mainland or to another island having a related species of its own, and no interbreeding results. It seems reasonable to suppose that immigration had occurred repeatedly, and that the migrants have become established only after the development of physiological isolation due to the previous intrusions. On the other hand, it is possible that certain forms of physiological isolation may occasionally arise as by-products of the adaptation to the environment. Thus, adaptation to different food plants

or soils may engender ecological isolation; adaptation to different climates may lead to a divergence of the breeding seasons (temporal isolation); physiological changes of various kinds may affect the sexual behavior, recognition marks, or smells, and give rise to sexual isolation. The basic problem which remains to be settled is how frequently and to what extent can the isolating mechanisms be regarded adaptational by-products arising without the intervention of the special selective processes postulated above. Only experimenal data could elucidate the situation further.

A very remarkable fact long known to taxonomists is that species in different groups, even among the sexually reproducing organisms, appear to be unlike entities. Thus, the morphological gaps between related species may be large or (as in Drosophila) very small; some species are variable and highly differentiated, others are clear-cut and uniform; the bar to interbreeding may be ecological, or due to sexual isolation, or to the structure of reproductive organs, or to hybrid sterility or to a combination of these and other causes. The unlikeness of species has led some biologists to abandon the search for properties common to all species—an example of faulty thinking. A dog, a bat, and a whale certainly do not look very similar, and yet zoologists recognize them to be members of the same class—mammals. Isn't it a task of science to detect fundamental similarities concealed by apparent unlikeness? A fundamental common property of species is the presence of isolating mechanisms. The very fact that isolating mechanisms are as diversified as they are is strong evidence for the prevention of interbreeding being an essential characteristic of the process of speciation. The precise means whereby the interbreeding is eliminated are immaterial so long as the exchange of genes is precluded. Any gene that raises an effective barrier to the mingling of incipient species is adaptively valuable, and hence may become the basis of speciation.

SUMMARY

By speciation is meant the fixation of discontinuity among organisms. Discontinuity is maintained by isolating mechanisms that prevent the interbreeding of carriers of different adaptive complexes of genes. A theory is suggested according to which the development of isolating mechanisms follows, rather than accompanies, that of the adaptive complexes themselves. The development of physiological isolation takes place principally along the geographical boundaries separating the distribution areas of the incipient species. Some evidence for and against this theory is discussed.

LITERATURE CITED

Dobzhansky, Th.
1937a. AM. NAT., 71: 404–420.
1937b. "Genetics and the Origin of Species." Columbia University Press, New York.
Dobzhansky, Th., and P. Ch. Koller
1938. *Biolog. Zentralbl.*, 58: 598–607.
Fisher, R. A.
1930. "The Genetical Theory of Natural Selection." Clarendon Press, Oxford.
Goldschmidt, R.
1932. *Proc. 6th Internat. Cong. Genetics*, 1: 173–184.
1933. *Science*, 78: 539–547.
Huxley, J. S.
1939. *Bijdragen tot de Dierkunde*, 27: 491–520.
Karpechenko, G. D.
1928. *Zeits. Ind. Abst. Verebungsl.*, 48: 1–85.
1935. *Theoretical Bases of Plant Breeding*, 1: 293–354.
Kozhevnikov, B. Th.
1936. *Biolog. Zhurnal*, 5: 727–752.
Meise, W.
1928. *Jour. Ornith.*, 76: 1–205.
Stern, C.
1936. AM. NAT., 70: 123–142.
Sturtevant, A. H.
1938. *Quart. Review Biol.*, 13: 333–335.

OBSERVATIONS ON THE ECOLOGY AND NATURAL HISTORY OF ANURA

I. HABITS, HABITAT AND BREEDING OF *BUFO COGNATUS* SAY[1]

ARTHUR N. BRAGG

Introduction

It has been some time since an adequate study has been made concerning the natural history of any single species of North American toad. Miller (1909) published an extensive account of the natural history of the American toad (*Bufo americanus americanus* Holbrook) based upon observations made in central Massachusetts. Some years later, Wright (1914) studied the same form in central New York. The life-histories of many of the eastern species have also been given by Wright (1931). Except for studies on feeding habits, short articles and incidental notes, these are the latest papers which have come to my notice.

The natural history of the western species of toads is quite inadequately understood. After the settlement of the central and western plains, biological workers in these regions quite naturally concerned themselves first with biological surveys and with the attendant problems of taxonomy and distribution. Such studies are still necessary and many are in progress, especially in the more sparsely settled areas, but it seems now that sufficient knowledge has become established for some studies to be directed more specifically toward an understanding of the lives of the individual animals themselves as they occur in their natural habitats.

It might be supposed by those unfamiliar with the environmental conditions encountered by animals inhabiting the plains that Miller's and Wright's papers on the American toad would give an adequate basis for understanding the habits of American toads in general. Indeed, Over

[1] Contributions from the Zoological Laboratory of the University of Oklahoma, No. 197.

(1923), speaking of *Bufo cognatus* in North Dakota, remarks, "Its . . . life habits are the same as those of the common toad" (*B. a. americanus*). It is one purpose of this paper to show that this is far from the case, at least in central Oklahoma. The adaptations to specific environments in the drier Southwest offer much of interest and value in comparison with those of the moister and cooler eastern section of the United States. In ecological terms, it is to be expected that habits which adjust a given species to complexes of environmental conditions in the grasslands biome will be different, both qualitatively and quantitatively, from those in the climax beech-maple forests of the East. These ideas were hinted in an earlier paper (Bragg, 1936) in which it was shown that the distribution of species in the three principal American families of Anura (*i.e.*, Ranidae, Bufonidae and Hylidae) follows closely the major climatic regions of the country.

Some examples of the differences in breeding habits of Anura in the East and of those in the western plains will make this point clear. *Bufo a. americanus* breeds fairly early in the spring with the crest of this activity about April 30 (Wright and Wright, 1933). The two common toads (*Bufo cognatus* and *B. woodhousii woodhousii*) of central Oklahoma have a breeding season extending well into the summer, and both of these usually breed only after rain. In any one year and at any given place, neither of these toads will breed in any numbers if rain does not come, even though there be plenty of water. Nor are these differences limited to toads. *Rana pipiens pipiens,* the common leopard frog of the East, begins breeding when the water temperature reaches 43–45 degrees F., with the crest of the period reached at 50–65 degrees F. (Wright, 1920). These temperatures are usually attained in New England at about April 1, but eggs of this species have been collected in Maryland as early as the middle of February in some years. In contrast to this, *Rana sphenocephala,* the southern leopard frog, which is the ecological equivalent of *R. p. pipiens,*

breeds about Norman, Oklahoma, at any time from late winter to late summer. I have seen them breeding or have collected their eggs in almost every month from February to August and at air temperatures ranging from about 40 degrees F. to over 100 degrees F.

Similar contrasts might be made between many ecological equivalents from these two general regions of the country, but nowhere are they more strikingly shown than among the semi-terrestrial Amphibia—and, of these, the toads (Bufonidae) are of special interest.

For several years, I have been observing the habits of the Amphibia, more especially of the toads, in central Oklahoma, principally during the breeding season. In the course of this study, which necessarily has been largely seasonal, one species has been emphasized, namely, the Great Plains toad, *Bufo cognatus* Say. It is the purpose of this paper to summarize the existing knowledge of the natural history and ecological relationships of this toad and to report observations made upon it mostly during the spring and fall of 1938. I know these toads only as they occur in the vicinity of Norman, Oklahoma, and all statements made which are unsupported by reference to the literature are to be interpreted as applying specifically to this area. The study is based upon extensive observations in the field at all hours of the day except between 2:00 and 8:00 A.M., supplemented by studies of the eggs, the embryos, the process of hatching and the process of metamorphosis made in the laboratory in conjunction with the studies in the field. The view-point has been that field observations are necessary if one is to understand the lives of the toads in nature, but that certain phases of the life history can best be observed in the laboratory, where equipment can be more easily used and measurements more conveniently made.

ACKNOWLEDGMENTS

I am indebted to practically the whole staff of the Department of Zoology of the University of Oklahoma for

various minor aids in the preparation of this paper. I especially wish to acknowledge the clarification of ideas received from discussions with Dr. A. I. Ortenburger. Mr. Albert H. Trowbridge, now of the U. S. Biological Survey, and Dr. Charles Smith have generously allowed use of their field notes on *Bufo cognatus* in Oklahoma. Dr. J. Teague Self has reported to me several interesting observations made while in the field for other purposes; and, finally, Dr. A. O. Weese has aided in the preparation of illustrations.

History of Bufo cognatus, Its Range and Habitat

Bufo cognatus was named and described briefly by the early American naturalist, Thomas Say, who found it on the Arkansas River in what is now Prowers County, southeastern Colorado. (For the original description, see Long, 1823.) Other early naturalists found it on the Plains (Holbrook, 1842; Baird, 1859; Yarrow, 1875; and others) and in later years scattered records of its occurrence have come from most of the states of the Great Plains (see Fig. 1).

Its general distribution includes the following states: Minnesota, North Dakota, South Dakota, Montana, Wyoming, Nebraska, Kansas, Colorado, Utah, Oklahoma, Texas, New Mexico, Arizona and California. It has also been taken in Mexico south of the Texas border. However, its range does not include all portions of each of these states. Strecker's papers on several of the counties of eastern Texas do not record this species. No authentic record is known from Arkansas (Black and Dellinger, 1938) and it does not occur in eastern Kansas, so far as known (Smith, 1934).

The first record from Oklahoma which I have found is that of Baird and Girard (in Marcy and McClellan, 1854) who figure a specimen collected in southern Oklahoma just east of the Wichita Mountains. So far as now known, the species occurs rarely if at all in eastern Oklahoma. It was

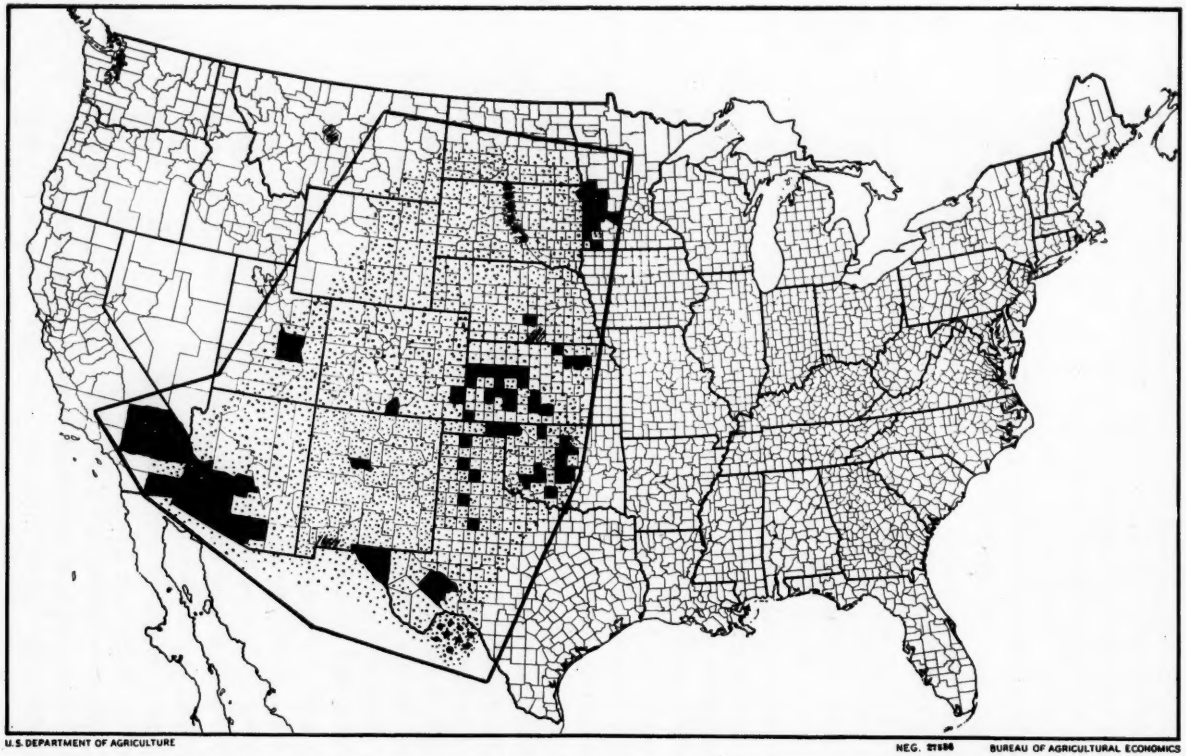

FIG. 1. General distribution of *Bufo cognatus*. Solid black indicates county records; large dots, records in the general area shown; cross-hatching, records considered doubtful, either as to fact or as to county; stippling, general range; black line bordering the whole, the probable area in which *B. cognatus* is likely to occur in proper habitats. All records outside Oklahoma were compiled from the literature cited; in Oklahoma, the specimens in the University of Oklahoma Museum of Zoology and personal observations supplemented the literature.

not collected by Trowbridge (1937, 1937a) in the southeastern part of the state and had not been reported east of Pottawatomie County a few years ago (Ortenburger, 1927). This is of interest because Pottawatomie County joins Cleveland County on the east, where all my observations have been made and where this toad is extremely abundant. Since the grasslands biome is interrupted by an oak-hickory associes in eastern Cleveland County, this definite break in the distribution to the eastward is to be expected. *Bufo cognatus* occurs in great abundance in central Oklahoma but more sparingly in the western portion of the state.

The northern limits of the species seem to be southwestern Minnesota (Breckenridge, 1938), the southwestern limits, the waters entering the Salton Sea in the Imperial Valley, California (Myers, 1930). The recent record from Minnesota suggests the presence of this form in northwestern Iowa at the present time, but Ruthven (1910) did not find it there. I have found few definite records from New Mexico, although Bailey (1913) lists this species as a characteristic inhabitant of his lower and upper Sonoran life-zones, which comprise most of the state. Cary (1917) lists it in his Sonoran life-zone in Wyoming but not in the transition zone of this state.

Within its general range, *Bufo cognatus* varies considerably in the number of individuals present in various fairly limited regions. For example, Ortenburger and Freeman (1930) did not collect this species in western Oklahoma, although they found *B. woodhousii woodhousii* to be fairly common. Similarly, Linsdale (1938) did not take it in his extensive survey of a limited portion of the Great Basin, Nevada. Ortenburger and Ortenburger (1926) saw it but rarely in Pima County, Arizona.

These observations are explained in part by what is known about the habitat of this toad. Wright and Wright (1933) summarize this as follows: "grazing lands or agricultural lands of the Great Plains, along irrigating ditches, flood plains of streams and overflow bottom lands."

Ruthven (1907) found these toads common "at dusk about irrigation ditches, but not elsewhere" in a region climatically deficient in rainfall (Arizona). Strecker (1910) found them extremely abundant in low places on the plains of northwestern Texas during wet seasons. Ruthven (1932) collected this species on the flood plain of the Green River, eastern Utah. In contrast, Bragg (1937) reported this species as mostly confined to the *higher* portions of the prairies and seldom in the immediate vicinity of inhabited portions of the city of Norman, Oklahoma (see Fig. 2

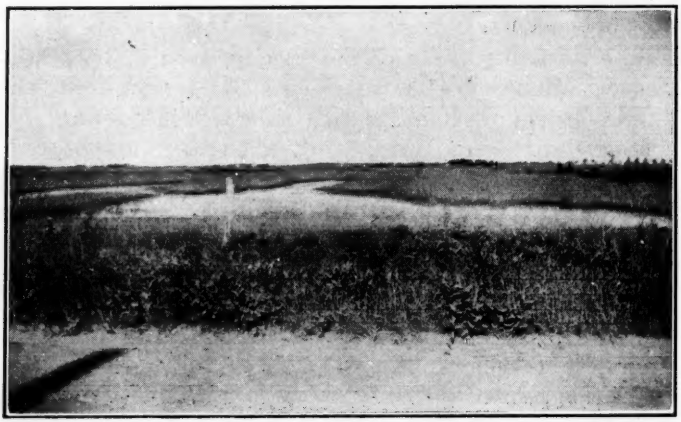

Fig. 2. A typical habitat of *Bufo cognatus* just south of Norman, Oklahoma. The field in the background has a large population of this species. In 1938, the toads bred in the flooded field shown in the middle distance.

for a typical habitat in this region). They have never been taken, so far as I can determine, on the extensive flood plain of the South Canadian River near Norman, even when present in enormous numbers on the prairie just above it during the breeding season. These observations are confirmed by Dr. Charles Smith, who tells me that the toads of a river-bottom farm in northern Logan County, Oklahoma, are all *B. w. woodhousii,* although, above it, on higher ground, *B. cognatus* is abundant. Brennan (1937) similarly found them only in the prairie habitat in central Kansas.

These differences in the observations of the various field workers seem to indicate that *B. cognatus* occupies different habitats in the different portions of its range. Since, in Oklahoma, these toads are quite susceptible to the influence of moisture, it is probable that the habitat occupied by them in any one region is largely conditioned by this factor. They seem to like it neither too wet nor too dry. They constitute the desert form in the Imperial Valley (Myers, 1930) but I can not agree with Myers that "*B. cognatus* is strictly a desert animal throughout its range. . . ."

From the bulk of the evidence available, it may be concluded that *Bufo cognatus* is primarily a toad of the grasslands biome which is able to extend its range into deserts of the Sonoran zone in limited numbers along irrigation ditches and similar low-lying areas where sufficient moisture is available. Under climatic conditions resulting in a mixed grass prairie, however, it tends to avoid the lower areas, thus automatically becoming excluded from woodlands and the flood plains of streams.

Taxonomy

The taxonomic relationships of *Bufo cognatus* have presented little difficulty. Some of the early workers considered this toad a subspecies of the old eastern species *lentiginosus* and, hence, refer to it as *B. lentigenosus cognatus* (*e.g.,* Yarrow, 1875; Cragin, 1881), but all modern authorities recognize it as specifically distinct. Cope (1875) reinterpreted the type specimen of *B. dipternus* Cope as a young individual of *B. cognatus.* Camp (1915) described a new form from Santa Paula, Calif., as a subspecies, *Bufo cognatus californicus,* as a result of which Say's toad automatically became *B. c. cognatus* (Say) and was so called by many herpetologists (*e.g.,* Storer, 1925; Ortenburger, 1927; Gloyd, 1929; Tanner, 1931). Since Myers (1930) clearly demonstrated that Camp's toad is specifically distinct from *B. cognatus* Say, the trinomial designation has seldom been used (Wright and Wright, 1933;

Smith, 1934). Hence, nearly all are now agreed that the correct name is the original one given it over a century ago, *Bufo cognatus* Say, with *B. dipternus* Cope the only synonym.

DESCRIPTION OF ADULTS

The recognition of *B. cognatus* is relatively easy (see Figs. 3 and 8). It is a medium-sized to large toad, the

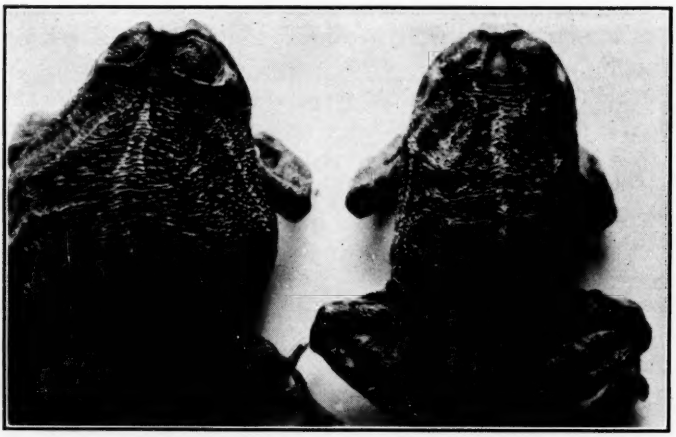

FIG. 3. Adults of *Bufo cognatus*, male on the right, female on the left.

females slightly exceeding the males in size. Wright and Wright (1933) give 47–95 mm and 60–99 mm as the lengths of the male and female adults, respectively. Smith (1934) records a female 114 mm in length. Boring and Liu (1934) indicate that some individuals may reach 115 mm. Thirty-five calling males, collected at Norman, Oklahoma, on March 28, 1938, ranged in length (after preservation in formalin) from 72–90 mm, with an average a small fraction above 80 mm. These figures indicate a size slightly larger than the American toad (54–110 mm) but smaller than *B. w. woodhousii* (56–118 mm) according to Wright and Wright (1933).

Its color is variable, but there is commonly a greenish cast to the dorsal and lateral portions; and when these are

not green, they are almost always gray or brown. The back is marked with large dark spots, greenish, dark brown or almost black and somewhat irregular in outline. These are usually arranged approximately in pairs, one of each pair on either side of a faint, light-colored mid-dorsal stripe. Sometimes the stripe may be absent or very faint. Occasionally one finds a specimen which has only a few spots, in which case they are small and scattered over the back (see Wright and Wright, 1933, Pl. XV, Fig. 3, for a typical example). Myers (1930) found that specimens from the region of the Pecos River, Texas, varied more in color and markings than is usual. On the sides are smaller spots, often broken up into vermiculations. The ventral surface is uniformly light, except that the vocal sac of the male forms a black, sooty-colored apron on the throat. This secondary sex character is very useful in distinguishing the sexes. On a few four-month-old females, I have observed a dark pectoral spot such as often occurs in *B. w. woodhousii* (Smith, 1934) and in *B. w. fowleri* (Wright and Wright, 1933), although some others examined lacked this character. It is interesting that I have never noticed this spot on adults, although I have handled hundreds of them.

One of the most characteristic and obvious features of the species is the presence of an osseous boss, just anterior to the eyes on the dorsal surface, from which two cranial crests extend posteriorly. These crests diverge from the boss in such manner that they commonly form a distinct V-shaped structure between the eyes, very conspicuous on old adults, and noticeably present on individuals just less than two inches long. Cope (1875) found that his young specimens (one and one half inches in length) lacked the crests, and I have specimens of this length which also have not yet developed them, although others but slightly larger show them faintly. Only occasionally are these crests less distinct than described. Therefore, they serve as an extremely useful character in the recognition of the species.

The parotoid glands are prominent, set wide apart, ovoid in shape, and extend obliquely posterio-laterad from just posterior to the eyes. The foot has two metatarsal tuber-

cles, each with a cutting edge, one of them larger than the other. These are used for burrowing.

GENERAL HABITS

This, like most American toads, tends to be nocturnal in habit. However, Strecker (1910) and Bragg (1937) have occasionally found them active during daylight. This is especially noticeable during moist weather and during the height of the breeding season. At times of prolonged drouth, especially when temperatures are high, these toads do not appear at all even during the night; at least, repeated search during such times has never produced a specimen in areas where they are known to be very abundant under other conditions. A light shower, however, will often bring them out in numbers.

When not active, these toads rest below the surface of the ground in burrows which they dig with the tubercles of the hind feet, the animal, in all probability, backing into the ground much as Scaphiopus is known to do and eventually becoming covered completely. The burrows have been little studied, for they are difficult to locate once the toad is below the surface. I have found but few during several years of observation; in one, the toad was below the heated portion of the upper crust and it seems probable that most burrows are deep enough for the animal to avoid excessive heat and to have moisture available. During breeding (which never occurs except when the ground is moist) the burrows are often quite shallow, mere depressions just fitting the body of the toad (Bragg, 1937). Whereas several toads may be found in such shallow burrows on a day succeeding a night of active breeding, on some occasions I have found none at all so occupied even after considerable search, in regions where I have found them before. Also, after a night of active breeding, adults which have entered a pool during darkness may spend the day in the pool. I have noted on several occasions spent or unspent females, males and mated pairs, separately or all together in the same pool. On the whole, it seems likely

that few individuals spent the day after breeding in either the pools or in shallow burrows as above described, for at several times when hundreds of toads have been seen in and about a series of buffalo wallows on an evening, none at all or but two or three have been found during the next day in either situation.

Breeding

The breeding places of *Bufo cognatus* are often quite restricted. According to Strecker (1910), they bred in temporary pools and small streams in Texas. In central Oklahoma, however, I have found them breeding only in temporary rain-formed pools, specially in so-called buffalo wallows.

These wallows, which occur abundantly in pastures, are shallow depressions of various shapes but often approaching a circle in outline. They were formed in earlier days by the rolling of bison, although cattle now often form similar wallows (Barkley and Smith, 1934). Such wallows are being formed at the present time in the buffalo pasture in the Wichita Mountains in southern Oklahoma. Some of the smaller ones are but a few feet in diameter, but larger ones may measure twenty or more feet. Two of them, measured on May 26, 1938, near Norman, Oklahoma, had the following dimensions:

(1)

North-south diameter	23 feet.
East-west diameter	20.5 "
Depth	5.5 to 9.0 inches (average, about 7 in.).

(2)

North-south diameter	9.5 feet.
East-west diameter	9.25 "
Depth (maximum possible)	10.0 inches (in center).
Depth of water at this time	8.5 " " "

The buffalo wallows are available to toads only in areas which have not been cultivated, for it is obvious that plowing of the land will completely destroy them. Under primitive conditions, they must have been much more numerous than at the present time and probably form the

natural breeding sites for the Great Plains toad in central Oklahoma. Typical buffalo wallows are shown in Fig. 4.

At present, the toads inhabiting cultivated areas often

FIG. 4. Buffalo wallows just northwest of Norman, Oklahoma (station 1). *Bufo cognatus* bred extensively here in the spring of 1938.

use flooded fields for breeding (Fig 5). I have found them in fields planted to rye or wheat but always only in the shallower portions of the pools. But once have I found a *B. cognatus* in deep water in a grain field, and this was a male actively swimming toward a chorus of this species in shallow water near the bank.

Bufo cognatus will also breed in shallow water along the edges of larger temporary pools, not located in cultivated fields; but only if the water is not muddy. One such pool (Fig. 6), just northwest of Norman, has been formed by three embankments where a road crosses an interurban track and a railroad running parallel to each other. The embankment for the road constitutes a dam to the south and the water settles above it, being held to the east and west by the embankments of the two tracks. The pool so formed is quite extensive (172 by 52 ft.) especially after heavy rains in the spring. It is about two feet in depth

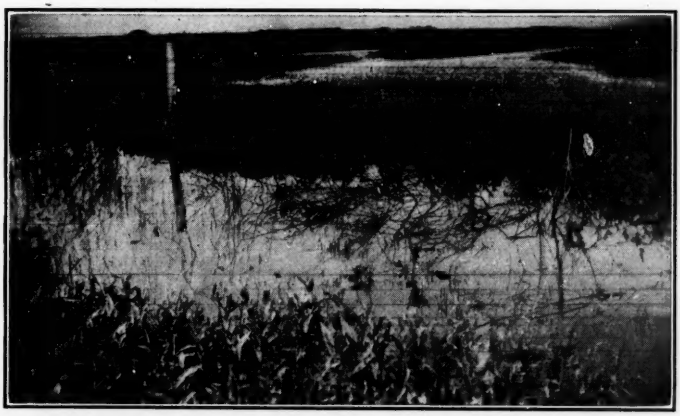

FIG. 5. Flooded ditch with a flooded field in the background. Picture taken during the height of the breeding season, 1938, just south of Norman, Oklahoma. This site has been watched closely since 1934 by Mr. A. H. Trowbridge, by myself or by us both and no evidence has been found that *B. cognatus* ever uses the ditch for breeding, although the field was used extensively in 1938.

in the southwest corner (varying, of course, with the amount of rain) and for some distance along the west side.

FIG. 6. The pool at station 4 looking northwest. Great numbers of *Bufo cognatus* eggs were laid in the northern end of this pool in 1938, but no larvae nor emerging young were found in the deeper water in the foreground.

To the east and north, it becomes progressively more shallow except at the south end, the east side of which is but slightly less deep than the west.

Bufo cognatus used this pool extensively during the spring of 1938 for breeding, but only the shallower portions of it. Even fully grown tadpoles avoided the deeper water, for I took relatively few from the southern portions of the pool at times when they were very abundant in the shallower portions. Also, at metamorphosis, thousands of young emerged from the water at the northern end and along the northern half of the east and west sides, but not a single specimen was ever found emerging from the deeper water near the southern bank.

In the region studied, *B. cognatus* rarely lays its eggs in muddy water, although I have occasionally found the tadpoles in fairly roily pools in wheat fields. I have never known them to breed in the relatively permanent pools, known as "tanks," in pastures even when present in large numbers about buffalo wallows close by. They do not breed in these even after spring rains which have been insufficient to fill the buffalo wallows, although the other common bufonid in this region, *B. w. woodhousii,* does so. Muddy ditches attract males, for I have seen dozens of calling males about them. However, I have never found eggs or tadpoles in ditches nor any evidence of their having been there. Once I found a mated pair in a ditch, but repeated search subsequently showed no tadpoles of this species here. I suspect that the depth of water is usually too great in ditches for breeding to occur.

The breeding habits of this species are most interesting. According to Wright and Wright (1933), the breeding season extends from April to September (May to July in the northern portions of the range) and is dependent upon rainfall. In central Oklahoma, I have found them breeding from March 29 to June 17, dependent not only upon rainfall but also upon temperature. This is shown by the following observations made during the spring of 1938.

On February 27, pools were full of water from recent

snow and rain. As might be expected, no toads were breeding. On March 4, the air temperature reached 78° F. during the day and was 64° F. at 7:15 P.M. The pools were full of water, but no toads appeared about them up to 10:30 P.M., at which time the temperature was 60° F. Some of the other Amphibia were breeding, but none of the bufonids of the region were included. For some time thereafter, daily temperatures were variable, often reaching or exceeding 70° F., but the toads did not breed. Meanwhile, the water in the pools nearly all disappeared through drainage and evaporation. On March 26, over two inches of rain fell, extensively flooding fields and filling pools. That evening the temperature was 9° C. No toads were out. The next day was marked by intermittent showers and a slightly rising temperature. Scaphiopus males were calling in some numbers at 9:15 P.M., as was also a species of Pseudacris. One male of *Bufo cognatus* was found beside a ditch. The temperature at this time was just less than 12° C., and the animal was slow in its movements as though stiff with cold. This was the only Bufo seen. The next day, March 28, it rained hard all the forenoon but ceased some time after noon; over four inches of rain fell. Beginning in the early morning and lasting throughout the day, Anura of several species could be heard calling. That evening, *Bufo cognatus* were out in large numbers, calling from flooded fields, ditches and buffalo wallows. Over four hundred were collected just west of Norman by a party from the university in less than one and one half hours; there must have been thousands of individuals within the township. The air temperature at 10:20 P.M., taken in the field just west of Norman, was 12+° C. At 1:00 A.M. it was just less than 14° C. It is significant that six people, all collecting or studying toads in the field about Norman that night, all found females to be quite rare. I saw but three, none of which was in the water with the males. Dr. J. Teague Self found a single mated pair, the female hopping between buffalo wallows carrying the male on her back. No eggs were in the pools at

4 P.M. and later the following day, but that evening occurred the greatest chorus of amphibian voices heard hereabouts since the spring of 1935. Thousands of *B. cognatus* were in and about all favorable breeding sites, and mated pairs were found as early as 10:30 P.M. Eggs were numerous in several pools the next morning and, in some pools, eggs were still being laid during the afternoon. The temperature during the height of this breeding congress (11:30 P.M.) was 18.5° C.

From these observations it appears (1) that *B. cognatus* breeds only after rain; presence of sufficient water is not enough in itself to stimulate the toads to breed. This is to be emphasized, inasmuch as Linsdale (1938) found *Scaphiopus hammondii,* a species which also is commonly supposed to breed only after rain, breeding in fields flooded by mountain streams in Nevada at a place where no rain had fallen for some time. (2) *B. cognatus* breeds after rain only if the temperature is not too low. Twelve degrees Centigrade is apparently the critical temperature, with males slightly more sensitive than females in their response at or near this temperature. As a comparison, it is perhaps well to mention that very few *B. w. woodhousii* appeared during great abundance of the breeding of *B. cognatus* on March 29 and that all that were seen were males. Males of *Scaphiopus hammondii* were also much more abundant on both March 28 and 29 than were females. The critical temperatures for these species, therefore, while close, is not the same for all; that of *S. hammondii* is probably somewhat below 12° C., whereas that of *B. w. woodhousii* is above it.

On May 7, an observation was made which shows that *B. cognatus* will continue to call even if the air temperature falls considerably below that critical for their emergence for breeding, provided that they are already in congress when the temperature falls. A heavy shower fell during the night of May 6. The next afternoon (4:15 P.M.) a large and lively mixed chorus of *B. cognatus* and *B. w. woodhousii* was observed in a flooded field. The latter

toads were not numerous and called but intermittently. The air temperature near the site was 16° C. and the water temperature in an adjoining ditch was 20° C. Late in the afternoon, the weather suddenly turned cold. At 11:00 P.M. the air temperature was 5.5° C. in an unprotected place in the city and some time later it was 8° C. close to where the toads were calling. Water in the ditch continuous with the field had a temperature of 14° C. *B. cognatus* were still calling here in some numbers. The calls appeared distinctly lower in pitch than during the afternoon and the tempo was much slower. I recorded in my notes of this incident that the calls reminded me of an alarm clock which was just running down. The air temperature was considerably below that found to be critical for the appearance for breeding after rain, but the water temperature was probably not much below 12° C., since it was 14° C. in the deeper water of the adjoining ditch. A pair of toads seen laying in a buffalo wallow at 5:00 P.M., just before the temperature started to fall, finished producing eggs and left the pool despite the lowered temperature. Several other incidents of this type have been observed more casually.

From the observations, it appears that the toads will not start breeding below 12° C. but will continue if already in congress when the temperature falls considerably below this. I have had no opportunity to learn the lower limit —that is, how low the temperature must fall to stop a breeding congress already in progress. Since calling males stimulate each other, it seems probable that a small chorus would be stopped by a higher temperature than a large one.

Psychological differences in the sexes during breeding are suggested by my observations, although the facts may be explained as well, perhaps, on the assumption of the differential physiological effects of hormones. Whatever the cause, the result is a well-marked difference in behavior. The following summary of observations will bring out the essentials.

(1) Males always precede females to the breeding sites.

After rains and at the proper temperatures, toads of both sexes emerge from their burrows in large numbers at or just before dusk. The males go immediately to pools of water and start calling. Females usually remain away from the pools for some time. As the evening progresses, more and more males congregate about certain pools and add their voices to the din. In the meantime, some of the females often migrate into adjoining pools, where they may remain for an hour or more, apparently paying no attention to the clamoring males. Later, usually beginning about 9:30 P.M., these females join the males, and mating actually begins (Bragg, 1936a). Some females, however, remain in the grass till about this time and then enter the pools where most males are calling, directly.

(2) Females apparently "prefer" larger pools; males do not. A male of *Bufo cognatus* will often call from a depression which contains a very small quantity of water; I have found them on many occasions calling from pools where, if eggs were laid, there would be little chance for the young to survive. But a male seldom succeeds in attracting a female to such a place. I have seen females actively hopping by calling males and within a few feet of them only to enter a near-by larger pool in which males were calling. Since males commonly call from the edges of pools (Bragg, 1937), occasionally a calling male will intercept a female in or near a small pool. Almost invariably, the female struggles as if in attempt to escape, sometimes succeeding in leaving the pool, taking the male with her. This is the probable explanation for the observation made earlier (Bragg, 1937) that eggs are seldom found in small pools, even after a congress of males has been about them. Sometimes, however, the female loses in the attempt to leave, in which case eggs may be laid. This probably explains, at least in part, why small pools, when they do contain eggs, seldom have more than one clutch each.

It is obvious that these reactions of the females constitute a definite benefit to the race. They insure that more clutches of eggs will be laid where they will have some

chance of developing before the water in the pool disappears through evaporation. In unscientific language, one might say that the female is more responsible than the male. The "problem of the male" is exemplified in the social activities of these toads in much the same sense as in mammals (Wheeler, 1934).

The reaction of the males to the mating calls of one another is also of interest in this connection. The first male to reach a pool starts calling. Other males arriving at this and adjacent pools join in. Inevitably, more males happen to gather about some pools than about others, and usually these are the pools of greatest extent. Within or about any one pool, if one toad starts calling, others immediately take up the chorus, both in this and in near-by pools. The result is that some pools are much noisier than others. As the night wears on, many males about the smaller pools cease calling and join those in the larger pools near-by, so that by 1 or 2 A.M. very few toads remain about the small pools, but the chorus in the larger pools has become greatly augmented. In this manner, large congresses are built up during the course of one night; but on another night, the height of the congress may develop at another favorable place under the same influences. Males are clearly stimulated by each other; and females, other things being equal, are attracted to the region of the loudest clamoring.

While the development of such congresses has been observed many times during the last three years, it was strikingly shown by some observations made during the spring of 1938. Just south of the main campus of the University of Oklahoma there is a polo field which becomes extensively flooded during heavy spring rains. Adjacent to this, on the southeast, is a low field bordered on the east by a deep ditch. On the night of March 28, after a rain of more than four inches, males of *B. cognatus* were very abundant here, calling from the flooded areas of the polo field, particularly in the southeastern portion at 9:15 P.M. At 12:40 A.M. there was less calling and fewer toads here, except at the very southeastern limit of the polo field by

a fence which separates this from the adjoining field. Most of the males had congregated here about a trough-like depression just north of the fence. On the next evening at about 10 o'clock, there were a few calling males in the polo field, particularly about this depression, and many more south of the fence, in the field adjoining. On March 30, from 10 to 11 : 30 P.M., there were no toads on the polo field, not even about the depression above mentioned, but a very large congress was present south of the fence. On subsequent evenings, the congress of toads moved progressively southward till eventually it was approximately 325 feet from the depression north of the fence where it had originally developed. Breeding was successful in both the depression and in the field farther south, for I collected metamorphosing young in both places late in May.

Amplexation occurs whenever a female comes in contact with a calling male, or with one which has just ceased calling. Males will also clasp other males but release them almost immediately. The reason for this is still unknown (see Bragg, 1937, for notes on this problem). The axillary embrace is used. Egg-laying is a long process, often lasting till well into the day following amplexus. Sometimes, however, it is completed within one night. The eggs are laid, a few at a time, typically in two long strings wound about on the bottom of the pool. The eggs are small (average diameter just less than 1.2 mm). Each is enclosed in a gelatinous capsule, and these capsules of each string of eggs are enclosed by a continuous, tough, elastic, gelatinous tube which serves to protect the eggs. There are about 20,000 eggs in each clutch, but this varies some with the size of the female. For more detail, see Bragg (1937).

At ordinary temperatures, hatching occurs in something over fifty hours (fifty-three in some laboratory cultures maintained in tap-water; Bragg, 1936a). Freezing weather, accompanied by snow, slows development but does not harm the embryos and young larvae in the field. At the opposite extreme, the embryos and larvae develop normally in pools which reach a temperature during the day

of 37° C. This is not necessarily the upper limit of tolerance but only the highest at which observations have been made.

Hatching

Hatching is a very interesting process. It has been observed in about one hundred embryos, but was studied most carefully upon fifty individuals brought in from the field just as they were about to hatch. The observations were made by means of a binocular microscope.

During embryonic development, the fluid-filled cavity of the egg capsule gradually becomes larger till it reaches 1.76 + to 2.19 + mm in diameter at hatching. For some time the embryo rotates slowly by means of its external cilia, but as the time of hatching approaches this movement becomes progressively slower and finally ceases altogether. The anterior end of the embryo is usually directed upward at this time and the head becomes pressed firmly against the wall of the capsule, which is thus forced against the retaining wall of the outer tube.

The embryo remains in this position for about ten minutes (sometimes less, often slightly more), but its cilia continue to beat. Suddenly, the membranes begin to bulge and a small circular opening suddenly appears in them at the point where the head is in contact with the wall. Immediately the embryo begins to emerge, coming slowly at first and with some apparent effort. The head of the emerging larva becomes distorted by being forced through an opening considerably smaller than itself. The opening gradually increases in size, partially by its edges becoming stretched, but it always remains smaller than the largest diameter of the larva. As the head comes completely free, there is a sudden jerk, caused by the elastic contraction of the edges of the opening as they reach the narrowing portion of the larva's body posterior to the head. The edges of the opening get caught, however, on the bulging posterior portion of the body, and another slow forced movement is necessary to complete the process of hatching. When the edges of the opening reach the narrowing caudal

portion of the animal, they again contract sharply and the larva is projected from the membranes into the surrounding medium.

As noted earlier, the head of the larva is usually directed upward. The animal is, therefore, forced from the membranes against gravity and begins immediately to sink downward. In so doing, it must come close to the membranes from which it has just emerged. In over sixty larvae observed, all but one caught upon the membranes by means of the sticky secretion of the adhesive organ. The larvae are enabled to do this because of the mechanics involved in hatching; the contractility of the edges of the opening in the membranes and the shape of the body of the larva are such that the body is turned at just the right angle as it is projected upward at hatching to fall back with the ventral surface of the head coming in contact with the membranes. Some larvae catch the membrane squarely by their ventral surfaces, but others catch it slightly to one side or the other. In the one exception observed, the larva swung too far to one side, failed to contact the membrane at the proper place, and fell to the bottom of the container.

The whole process of hatching occurs quite rapidly. Only one animal was observed to take longer than one minute from the time that the opening first appeared. The usual time is about one half minute. The exact time consumed appears to depend upon the size of the original opening, although this was not checked carefully.

Absolutely no muscular movements were to be seen in any of the larvae during hatching. I have seen muscular movements both before and just after hatching, so the muscles must be functional at this time. The fact that they are not used during hatching raises the question as to source of the considerable force necessary to push the larva through an opening which is considerably smaller than itself.

My observations show that three factors are involved, two of which are mechanical in the sense that no vital processes are used. These are (1) the elasticity of the

membranes, particularly that of the outer tube; (2) pressure against this elasticity by the accumulation of liquid around the embryo in the capsule; and (3) action of the cilia on the external surface of the body. This last factor is of much less importance than the other two. As evidence, I submit the following:

(1) The space about the embryo increases as development progresses and more fluid accumulates in the cavity of the capsule. (2) the outer tube appears to be very elastic whenever a portion of it is handled. (3) At hatching, there is always a violent contraction of the tube and of the edges of the opening in the tube through which the larva has just emerged, so much so in fact that, if the middle one of three adjacent embryos hatch, one has to look carefully to determine where the middle capsule has been, so closely do the other two embryos approach each other. In some cases one would hardly suspect that a larva had hatched from the appearance afterward. One can, however, often see faintly the wrinkled laminations of the collapsed capsular wall. Also, the whole egg-string for several millimeters on each side is shaken by the emergence of each larva.

The cause of the appearance of the opening through the tube which is utilized at hatching is still not certainly known. All conditions are just as one would expect if a frontal gland were to become active and its secretion were rapidly to dissolve the substance of the wall as described by Noble (1926) for other species.

The percentage of hatching is high under favorable conditions in the field. Of more than fifty clutches observed, only one has been found in which most of the eggs did not hatch, provided the pool was not obviously contaminated. In the one exception, none of the eggs hatched, despite the fact that tadpoles produced earlier in the same pool were developing normally. In the laboratory, frequent changes of water are necessary to avoid injury from products produced by the disintegration of the jellies surrounding the embryos. Crowding in laboratory cultures kills many em-

bryos, perhaps through lack of oxygen, but if the eggs are allowed to remain as left by the parent toads in the pools, they usually thrive.

The variations in developmental rate observed earlier in laboratory-grown embryos (Bragg, 1938) are also evident in embryos growing undisturbed in the pools; but the variations are not so marked and striking. It is fairly certain, therefore, that these embryos, like so many other aquatic animals, can withstand great changes in environmental conditions but are, at the same time, influenced by relatively minute changes. It is also evident that some variation in rate of development is inherent in individual embryos, as maintained earlier.

FACTORS LIMITING NUMBERS

Almost nothing has been published concerning the ecological factors, either physical or biotic, which tend to limit the numbers of individuals of *Bufo cognatus*. As applying to the American toad, Miller (1909) estimated that 85 per cent. of the young are killed as follows:

Non-fertile eggs	15 per cent.
Drying of pools	25 per cent.
Predators	39 per cent.
Fungi	1 per cent.
Other diseases	5 per cent.

Thus, about 15 per cent. of the eggs produced eventually passed through metamorphosis. Of these, 20 per cent. were killed by drying; 25 per cent., by the rigors of winter; 15 per cent., by becoming trapped in sewers and wells; and the remainder by various causes.

General observations indicate that these percentages would be somewhat different for *Bufo cognatus*. Few non-fertile eggs have been found, and my general impression is that 15 per cent. would be too high. The drying of pools, however, is a most important factor in the death of eggs and larvae. For example, during 1936 and 1937, no toads of this species succeeded, in the vicinity of Norman, in effective breeding because of this factor alone, although

at least some eggs were laid during the spring of each of these years. In 1938, tadpoles in smaller pools met a similar fate, but those in the larger pools had ample time to pass metamorphosis. I have no clear idea as to what percentage of young so destroyed would be over a long term of years, but I am certain that it would greatly exceed 25 per cent. This is, of course, what could be expected in the drier habitat of central Oklahoma, as compared with central Massachusetts, where Miller's work was done. The comparative scarcity of these toads in still drier regions (*e.g.,* western Oklahoma, Arizona) may well be accounted for in large part by the failure of breeding during most seasons to be effective because of this one factor.

Predators play an important role in reducing the number of tadpoles. The spadefoot toad, *Scaphiopus hammondii,* often breeds in buffalo wallows. Its tadpoles are much larger, more active and develop at a much faster rate than those of *Bufo cognatus;* and they are carnivorous. Trowbridge and Trowbridge (1937) presented indirect evidence that these large tadpoles feed upon the smaller *Bufo* larvae, but they had never seen a direct attack. On several occasions, I have seen a Scaphiopus larva attack, kill and devour a tadpole of *B. cognatus,* and in one instance believe them chiefly responsible for the complete disappearance of *Bufo* larvae from a pool. Predaceous beetles also take a heavy toll. *Hydrophilus triangularis* invaded the buffalo wallows in large numbers in 1938, and its larvae were seen feeding upon tadpoles. At present, these are the only known predators, but I have reason to suspect other animals. I have several times found the terrapin (*Terrepene ornata*) in and about the breeding pools, but never was able to prove that it ate either eggs or larvae. The nature of the breeding sites precludes the possibility of fishes, most other amphibians, or aquatic reptiles as predaceous enemies.

During metamorphosis, the tiny toads are quite defenseless, and one would expect them to fall prey to many animals, especially to birds. Dr. Charles Smith informs me

that he has seen crows picking up young toads from the edges of buffalo wallows on two occasions. Since no other toad in central Oklahoma uses buffalo wallows extensively for breeding, it is very probable that these were *B. cognatus.*

I have never seen evidence of fungous or other diseases attacking eggs or larvae; but fecal contamination of the water in overgrazed pastures sometimes kills all the eggs produced in a given pool (Bragg, 1937).

Little is known about the enemies of adults. Dr. A. I. Ortenburger informs me that the hog-nosed snake (Heterodon) is very fond of toads. Since snakes of this genus are common in the habitat of *B. cognatus,* it is probable that some individuals fall prey to them. Black snakes and bull snakes eat *B. w. woodhousii* and, very likely, *B. cognatus* also. Dr. Charles Smith recently shot a large hawk which had just caught a toad that it was eating. The victim, a female of *B. w. woodhousii,* was still alive. It seems likely that hawks might also catch *B. cognatus,* particularly those active in the daytime.

The automobile kills hundreds of toads each year. I have seen as many as fifty dead toads along a mile of paved road after a rain. Many are killed also in the unpaved country roads about Norman, especially during the breeding season.

The Great Plains toad, like its eastern relative, often becomes trapped in pits from which it can not escape. In one case, more than twenty-five were taken from a well-like depression. I have also found them trapped in post-holes. It is doubtful if they often fall into sewers, as does the American toad, because they frequent human settlements very little.

Apparently, very few of these toads are killed by winter. At least, thousands were present during breeding in the spring of 1938 in a region where they had not bred successfully for at least two seasons. If winter-killing were common, one would expect the numbers of toads to be visibly depleted after three winters had passed without breeding.

The parasites of *B. cognatus* are very imperfectly known. Trowbridge and Hefley (1934) found a single individual to harbor a heavy infestation of intestinal Protozoa (Opalina sp.). A cestode, referred to *Ophiotaenia magna,* was also found. No trematodes, nematodes or arthropods were seen. Dr. J. Teague Self has noted heavy infestations of intestinal Protozoa in several individuals. He has also found a cestode (*Nematotaenia americana* Jewell, *Distoichometra bufonis* Dickey, or a form closely related to one of these) which occurs commonly in the small intestine. More knowledge is badly needed upon the distribution, life-histories and the effects of parasites of toads.

(*To be concluded in the September–October, 1940 issue*)

RADIATION AND THE HEREDITARY MECHANISM[1]

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IN this symposium on "Radiation and Life," I have been assigned to talk about the influence of radiation on the hereditary mechanism. By this we mean effects on genes, chromosomes and on the chromosomal mechanisms accompanying meiotic and mitotic cell division, which form the visible basis of the phenomena studied in genetics. It has been found that these structures are affected by all those kinds of radiations that produce ionizations, such as x-rays, radiations from radioactive sources and neutrons, which do not themselves produce ionizations, but which do so by virtue of the recoil protons arising in any hydrogenous material. Within wide limits it has been found that the type and the rate at which these effects are produced are independent of the specific kind of radiation and are only a function of the number of ions which these radiations produce in a given volume, so that for most purposes only the total dose of ions need be considered. In addition, ultraviolet light below 3,000 AU has been found effective, although, as we are now beginning to learn, its effects may differ qualitatively in some important respects from those produced by the ionizing radiations. The ultraviolet light in such experiments attacks proteins in their aromatic amino acids and nucleic acids in their pyridine groups. The ionizing radiations attack any molecule without preference. It is therefore plausible that the ionizing radiations should be able to cause a wider variety of types of effect.

Nevertheless, both the ionizing and the ultraviolet radiations should be grouped together in two important re-

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spects. First, their primary action is unphysiological. Their primary action consists in either destroying or changing the function of the molecule which they attack, in striking contrast to the action of light in photosynthesis or in visual perception. Second, they both can and generally do hit by an all or none law, in which a single ionization or absorption produces the entire macroscopic effect independent of all other ionizations and absorptions. This feature stands in contrast to normal physiology where we invariably deal with large numbers of molecules of each kind, and where the elimination of a single molecule could therefore never result in observable effects. It is, however, intimately related to the unique position of the genes in cell physiology, for which it appears to be characteristic that each type is only present in one specimen per chromonema.

In order to appreciate the significance of this last point clearly, let us consider a crude model: A cell may contain a thousand molecules of a certain enzyme which may be connected with its respiration or other metabolic functions, and let us assume that one half of these molecules, 500, would have to be knocked out in order to render the cell non-functional. Let us assume further, that the cell contains 10 "genes," that is, molecules, perhaps in size similar to the enzyme molecules, but only one specimen of each kind, each having a different function in the cell, so that a change in any one of these genes will change the entire cell; for simplicity of discussion we will say that all changes are lethal. Let us now irradiate this cell with an "unphysiological" radiation. Let us choose a dose which knocks out one fifth of the molecules in question, *i.e.*, there is a chance of one in five for each molecule to be knocked out. The effect on the enzyme system will be negligible, only 20 per cent. being eliminated. That may show up for a while in the metabolic activities, but it is not a fatal injury. Therefore, no cells will be killed by the action of this dose on the enzyme system. On the other hand, the chance that in a given cell no gene will be hit, is

$$(1-\tfrac{1}{5})^{10}=.13$$

That is, only 13 per cent. of the cells will have survived this dose because of the action of the dose on the genes. The survivors will have their genes quite unimpaired. Note that this is not caused by a greater sensitivity of the genes as such, but merely by their unique standing in the cell organization. Let us see how the survival fraction will depend on the dose. For each individual molecule the probability of not being hit will decrease exponentially with increasing the dose. This is a simple consequence of the quantized nature of the elementary processes involved. For the cell as a whole we will see, therefore, that the survival fraction decreases exponentially, only with a ten times greater decay-constant, corresponding to the ten times greater sensitive volume of the cell as compared with that of the individual gene. For sufficiently small doses, the number of cells killed by the action on any of the genes will be proportional to the dose. There will be no threshold value; even the smallest doses will kill a correspondingly small fraction of the cells. Only for very large doses, when a considerable fraction of all molecules are harmed, will there be interference with the normal physiological reactions involving large numbers of molecules. Then the exponential law of decrease of survivors will be replaced by a threshold effect, the remaining cells being finished off uniformly.

This peculiar type of dose-dependence of the harmful effects, an all-or-none law involving increasing fractions of the cells, brings the radiation effects on the hereditary mechanism in a class with still other types of phenomena: the inactivation of enzymes by radiations, of viruses, of bacteriophages, and, most important, the killing of bacteria. This was shown by experiments of R. Wyckoff (1930; 1932), of A. Hollaender (1936) and of Haines, Coulson and Lea (1937), for all types of ionizing radiations and for ultraviolet light. Hollaender and Emmons (1939) have even succeeded in producing mutant forms of a spore-forming non-sexual fungus. For enzymes, viruses and bacteriophages all this is readily understandable;

these represent single molecules themselves, while the results for bacteria prove that these cells also contain gene-like centers in the sense that they can not function if certain individual molecules are rendered non-functional. It appears that these conclusions for bacteria are borne out by a still wider class of experiments, namely, the killing of bacteria by disinfectants or by heat. Also by these agents, the first effects appearing upon small "doses," is a killing of a dose-proportional fraction of individuals and with no effect on the rest. This again must be interpreted as interference with controlling individual molecules governed by statistical laws. These implications of a large body of hitherto somewhat uncorrelated observations have recently been emphasized by P. Jordan (1939) in several important publications.

Now let us begin at the beginning with the simplest case, the inactivation of an enzyme by ultraviolet light. The best work on this problem was done by Kubowitz and Haas (1933), who investigated the hydrolytic enzyme urease, the molecular weight of which lies around 400,000. They found that this enzyme was inactivated in a wide range of ultraviolet, from 1,960 AU to 3,660 AU. In this range the absorption coefficient changes by a factor 5,000, the inactivating rate being nearly proportional to the absorption coefficient. The quantum yield of inactivation, however, is not equal to one but is quite small, about one in 250, *i.e.*, on the average, only one out of 250 absorbed quanta inactivate a molecule. It appears that the absorption of the protein in this range is predominantly due to the absorption of the aromatic amino-acids. The primary effect may consist in the splitting of the adjacent peptide bond, since we know from a model experiment of D. C. Carpenter (1939) that this can occur. These results should be interpreted to mean that of the many amino acids present, only one or a few are situated so that an absorption by them entails inactivation of the enzymic capacity. In the region around 2,600 AU, where most of the other UV-work has been done, this requires a dose of about $2 \cdot 10^6$ erg/cm^2 for the destroying of half the activity.

In the case of pepsin, the measurements of Gates (1934) indicate that a bigger dose of $5 \cdot 10^7$ erg/cm^2 is required for half inactivation. Pepsin is a very much smaller molecule than urease, and it may be that its greater resistance is connected with its smaller size.

In the case of a virus we have the measurements by Hollaender and Duggar (1936) on the inactivation of the tobacco-mosaic virus. Here the dose for half inactivation is only $1.6 \cdot 10^5$ erg/cm^2. In comparing this with the inactivation of enzymes we must, however, bear in mind that the effect measured by the experiment is a different one, namely, the loss of the capacity to produce lesions in the host and that probably means the loss of the capacity to reproduce. It is indeed very plausible that this capacity for reproduction should be more vulnerable than the enzymatic faculty. The latter depends on the activity of selected areas in the molecule, and a change in a distant part may not interfere with it. Whereas in reproduction, the entire molecule is necessarily involved and its vulnerability should then increase in proportion to its size. I have found similar values for a bacteriophage, which is in keeping with the close relationship between these two types of particles.

With bacteria and ultraviolet light several authors have done valuable quantitative work—Wyckoff (1930; 1932), Hollaender and Claus (1936) and Herčík (1937). They all find the exponential decrease of the survival fraction, and a dose of about $2 \cdot 10^4$ erg/cm^2 to kill half of the irradiated sample. This is again a considerably smaller dose than that required for viruses but is not, by far, in proportion to the very much greater size of these organisms. It can be explained by assuming that these cells contain a number of controlling particles of virus-size. Hollaender and Duggar (1938) in a most interesting series of experiments have also been able to detect and to analyze some apparently purely physiological effects of ultraviolet light on bacteria. The main effect is an extension of the normal lag-period. It is measurable only with doses that leave but a small fraction of the organisms alive. Very prob-

ably we have here before us the direct effects on some enzyme system, involving large numbers of molecules, so that the effect is dose proportional in each cell.

Up until now we have confined our attention to an analysis of effects of ultraviolet light. The effects consisted almost exclusively in destruction, inactivation of enzymes, of viruses, and killing of bacteria. In view of what we shall see later about the effects of radiation on genes I think it is probable that the limitation to these extreme effects is solely due to the technique of detection. In reality, there should also here be many cases of "viable mutations." The experiments mentioned so far afford proof of the importance of individual atomic groupings for the whole organization and the comparison of the absorption spectrum with the inactivation-spectrum can sometimes give a hint as to the nature of the primary photochemical process. An essential prerequisite to this success was the fact that these objects are so small that they absorb only a little of the impinging radiation. The intensity of radiation at the sensitive points is therefore equal to the outside intensity and can be evaluated with accuracy, and the relation between dose and effect can be worked out quantitatively. For these reasons ultraviolet work is more rewarding than the work with ionizing radiations on these objects and much can be hoped from further refinements in method and from collaboration with photochemistry.

When we turn to higher organisms, the situation changes with respect to all these last-mentioned points. We lose the possibility of measuring the intensity of the ultraviolet light at the sensitive points because it is not sufficiently penetrating. In most cases, it is not even possible to get any effective doses to the critical points. For these reasons the ionizing radiations have been more important for the study of effects on the hereditary mechanisms. For these radiations there is no analogue to the spectral analysis which is so helpful in the case of ultraviolet light, but this lack of finesse on the part of the physical agent is amply made up for by the infinitely greater

variety of phenomena that are produced on the biological side.

The macroscopical effects that the experimenter can observe are all concerned with the nucleus of the cell. They can be seen either by direct inspection of the irregularities produced in the chromosomal mechanism of the irradiated cell or of its offspring, or, indirectly, by a genetic analysis of the F_1 or F_2 of irradiated parent-individuals or parent-gametes. It is very important to bear in mind that even the apparently most direct effects of radiation, those observable in the irradiated cell itself, are a long way removed from the initiating ionizations. Indeed, the atomic physicist would class anything the cytologist can see with his instrument as macroscopic.

The first thing we have to decide for any given effect is its dependence on the dose. Is it an all or none effect, where the fraction of affected cells depends on the dose (in simple cases being directly proportional to it), or is it a graded effect, more or less uniform on all cells, where the grade depends on the dose. In the first case, we speak of a single hit effect (the hit need not be a direct one, *i.e.*, the ionization need not take place at the affected gene); in the second case, we speak of a mass effect. For bacteria we saw that the single-hit effects were easily measurable at doses at which the mass-effects were still small. In higher cells this is not necessarily so. If growing tissue is irradiated, it appears that nuclear divisions that were just about due to start are delayed by a time that is perhaps proportional to the dose (Carlson, 1938). This would seem to be a typical mass-effect. We might here assume that part of the substances controlling the division are destroyed, so that the rate of the reactions in which these substances take part is decreased. However, the very low dose (100 r and less) at which this effect is quite pronounced suggests a somewhat different interpretation, namely, that there are a very large number of parallel reactions each of which can be delayed, possibly each by a single hit, and that the observable delay in mitosis is a summative effect in the sense that each, sev-

erally, of the inhibitions have to be removed before mitosis can proceed. In this case we should expect that the delay is proportional to the logarithm of the dose, rather than to the dose itself. The experimental data are not yet sufficient to decide this point. This picture would link the effect to the typical single-hit cases.

As soon as the new mitoses appear, a variety of aberrations can be seen: broken chromosomes, broken chromatids, fusions of broken pieces, reciprocal translocations and still more complicated rearrangements in which more primary breaks are involved. The comprehensive and elaborate analysis by Bauer, Demerec and Kaufmann (1938) of giant chromosome rearrangements, of Sax (1938) on Tradescantia microspores, of Carlson (1938) on mitoses in the neuroblasts of a grasshopper, and of Muller, Makki and Sidky (1939) on dose-dependence of complex rearrangements in giant chromosomes of Drosophila have contributed much to a clarification of the sequence of events once the effect has increased from the atomic to the chromosomal order of size. About this intermediate phase we are still entirely dependent on conjectures.

The aberrations start as simple breaks of chromosomes or chromatids; the broken ends then have a strong tendency to recombine with other broken ends but not with free, unbroken chromosome ends. This recombination is limited in space and time so that in cases where many primary breaks are produced recombinations tend to occur in pairs and not entirely at random. However, at least in Drosophila, the spatial arrangement of the chromosomes in the sperm nucleus appears to vary sufficiently from cell to cell so that there is no preponderance of recombination of any specified region with any other.

The appearance or non-appearance of identical breaks in two sister chromatids has been taken as indicating whether the chromosome in question had already split at the time of irradiation. The argument was twofold: (a) since the break is caused by a single ion pair which can not act at two morphologically different points, sister-breaks indicate doubleness at time of irradiation, and

conversely; (b) in cases where only one of two sister chromatids is broken the lesion could not have applied to the original single chromosome. The application, however, of these criteria has led to difficulties. Sax (1938) found that sister-breaks and single breaks can occur in the same cell, even in the same chromosome. Although it is true that irradiation at early stages of the cycle causes more sister-breaks and vice-versa, the two phenomena overlap over a longe range, seemingly requiring a lack of coordination between different chromosome-regions in performing the duplication. Sax has therefore advocated the view that the first of the above arguments was invalid; sister-chromatids should both be breakable by the same single hit. I do not think that such a view is tenable on physical grounds. One might try to sacrifice the second argument (that a single chromatid-break proves doubleness at the time of irradiation). It seems natural to assume that if a chromosome is broken and doubles afterwards, one of the chromatids may reunite thus giving the impression of a single chromatid-break. In this case, however, we should expect a minimum fraction of single chromatid-breaks however early we irradiate, and this is not borne out by Sax's observations. At present, therefore, I see no alternative but to interpret Sax's findings on their face value—saying that the duplication really does proceed with little coordination. We would then have to assume that the comparative uniformity in the timing of the cycle in different cells was again the outcome of its dependence on many individual reactions.

With respect to deficiencies, Demerec, Kaufmann and Hoover (1937–38) have found a very important result. Making a statistical analysis of the lengths of deficiencies which are produced by x-rays, they find a bi-modal distribution indicating two different types of deficiencies. The long deficiencies, which are uniformly distributed over all lengths and should be attributed to two independent breaks, with elimination of the intercalary part—and the short deficiencies, involving only one or a very few bands and which on statistical grounds must be ascribed to single

hits. This latter class is interesting theoretically, since it seems to show how very far the effects of a single hit can spread on the chromosome. We should, however, bear in mind that this spreading is not an immediate effect. It may well be connected with the difficulties of coordination which must confront a chromosome, one gene of which has been rendered incapable of reproduction.

Demerec and Hoover (1937–38) have also recently contributed a very valuable cytological study of a large number of genetical changes caused by x-rays in a picked region of the X-chromosome of *Drosophila melanogaster*. They found all viable changes to be free of chromosomal aberrations and deficiencies. Also of the recessive lethals the majority showed no chromosomal rearrangements, although many of them showed some deficiencies—the majority belonging to the one-hit class. This result explains why it was possible to find the linear relationship between dose and mutation rate for these classes of mutations, since we see now that they are of a type which stands apart from the more complex effects which eliminate a considerable fraction of all irradiated cells before they can be analyzed genetically.

This brings us at last to the mutations proper. As you all know, it was the startling announcement by Muller in 1927 that large numbers of these could be produced by x-rays in Drosophila, and the independent work of Stadler with similar results in barley, which started off the vast amount of research of which I could only give you such a very deficient account. The first thing that could be established about the effect was its proportionality to the irradiating dose, and this, as we have seen, has survived the sometimes obscuring complexities of later and more elaborate research. The proportionality rule gave the basis for the single-hit interpretation and for the calculation of sensitive volumes (Timoféeff-Ressovsky, Zimmer and Delbrück, 1935). These sensitive volumes were found to be quite large on the atomic scale—comprising several hundred atoms even in cases where a well-defined mutational step is considered—like that which changes

the eye-color from normal to eosin, an intermediate shade. Recent experiments with neutrons by Timoféeff-Ressovsky and Zimmer (1938) support the view that these sensitive volumes represent well-defined areas and that the effect is really a direct one on the gene. Such large sensitive volumes could easily be explained by analogy with inactivation of enzymes which, as we saw, react similarly. We would say that the primary chemical change was rather unspecific in causing a given phenotype response, many "chemical mutations" showing the same phenotype. That would leave out of consideration, however, the important class of back-mutations, for which we have no analogue from enzyme-chemistry. They should be very much rarer than they have actually been found to be. Possibly a careful cytological investigation of such reverse mutations could throw more light on the problem.

For some time the relation between point-mutations and chromosome-mutations has been under discussion. The cytological analysis showed an apparently uninterrupted series from the gross rearrangements to the minute deficiencies which in the genetic analysis are not any more distinguishable from point-mutations. We now know at least that we have to distinguish between the two-hit and the one-hit cases. We also know that the one-hit cases may show visible, *i.e.*, macroscopic, changes in the chromosome. Another important distinction appears to emerge from the work of Stadler (1936) on the genetic effects of ultraviolet light in maize. His results show that ultraviolet light does not produce translocations. From this we conclude that it does not break the chromosomes. It does, however, produce deficiencies and mutations. For these the primary chemical change must therefore be different from those which produce breaks. These findings are corroborated by more recent work of Muller and Mackenzie on Drosophila (1939). But the question remains, whether all the one-hit mutations and deletions are of this macroscopic type or whether there is another subdivision between this type and such cases, where the

mutation proper is confined to a simple chemical change affecting only one radical, and which is propagated as such. At present we can not even give a tentative answer to this question because we are still so ignorant concerning the primary activities of the gene. We do not know what particular structural qualifications enable a gene to multiply on the one hand and to affect the phenotype on the other hand, if, indeed, these two functions can be separated. We know that for viruses and bacteriophages these qualifications must be quite exacting since their capacity to reproduce is so easily destroyed by radiation. But it is perhaps not permissible to infer that the same holds true for genes, since the reproduction of the gene is a normal function of the cell, whereas viruses and bacteriophages may have to enforce their reproduction by preliminary reactions, and it may be that it is these which are inhibited by the radiation. For instance, in the case of the bacteriophage we know that a prerequisite to its reproduction is its specific absorption into the host-bacterium. We will first have to find out whether or not the radiation is primarily interfering with this capacity to be adsorbed.

In giving you this brief survey of modern radiation work in biology, it has been my intention to emphasize the close interrelationship of the observed effects in a wide range of biological material. They put into striking relief the very peculiar organization of the cell in which the contribution of individual molecules plays an essential rôle. We see emerging here a new field of physiology, beyond that of enzyme-chemistry, which at present enjoys such splendid success in the hands of organic chemistry. In this new field a new feature, that of statistical fluctuations always attendant on small numbers of independent entities, must become of paramount importance. At present we do not know how the cell manages to exhibit such outward regularity in spite of the molecular incoherence. This problem will doubtless come into great prominence as we learn more about its details, and may require new conceptual ways of approach. But even as we enter this new territory, we are rewarded at every step with new insights into

the wonderful mechanics of the hereditary mechanism, for the exploration of which radiation has furnished a powerful tool.

LITERATURE CITED

Bauer, H., M. Demerec and B. P. Kaufmann
1938. *Genetics*, 23: 610.
Carlson, J.
1938. *Genetics*, 23: 596–609.
Demerec, M., B. P. Kaufmann and Margaret E. Hoover
1937–1938. *Ann. Rep. Dept. Genetics, Carnegie Inst.*, pp. 40–47.
Carpenter, D. C.
1939. *Science*, 89: 251.
Gates, F.
1934. *Jour. Gen. Physiol.*, 18: 265.
Herčík, F.
1937. *Jour. Gen. Physiol.*, 20: 589–594.
Hollaender, A., and W. Claus
1936. *Jour. Gen. Physiol.*, 19: 753–765.
Hollaender, A., and B. M. Duggar
1936. *Proc. Nat. Acad. Sci.*, 22: 19.
1938. *Jour. Bact.*, 36: 17.
Hollaender, A., and C. W. Emmons
1939. *Genetics*, 24: 75.
Jordan, P.
1939. *Arch. Ges. Virusforschung*, 1: 1.
Kubowitz, F., and E. Haas
1933. *Biochem. Zeits.*, 257: 337.
Lea, D. E., R. B. Haines and C. A. Coulson
1937. *Proc. Roy. Soc. London*, Series B, 123: 1.
Muller, H. J., A. J. Makki and A. R. Sidky
1939. *Jour. of Gen.*, Vol. 37, No. 3.
Muller, H. J., and K. Mackenzie
1939. *Nature*, 143: 83.
Sax, Karl
1938. *Genetics*, 23: 494–516.
Stadler, L. J., and G. F. Sprague
1936. *Proc. Nat. Acad. Sci.*, 22: 572–591.
Stadler, L. J., and F. M. Uber
1938. *Genetics*, 23: 170.
Timoféeff-Ressovsky, N. W., K. G. Zimmer and M. Delbrück
1935. *Nach. Ges. Wiss. Gött.* Fachgruppe, VI, N.F., 1: 189.
Timoféeff-Ressovsky, N. W., and K. G. Zimmer
1938. *Naturwissensch.*, 26: 362.
Wyckoff, R.
1930. *Jour. Exp. Med.*, 52: 769–780.
1932. *Jour. Gen. Physiol.*, 15: 351–361.
Zimmer, K. G.
1938. *Strahlen-Therapie*, 63: 517.
Zimmer, K. G., and N. W. Timoféeff-Ressovsky
1938. *Strahlen-Therapie*, 63: 528.

LONGEVITY IN *DROSOPHILA MELANOGASTER* AND ITS EBONY MUTANT IN THE ABSENCE OF FOOD

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INTRODUCTION

THE effect of the absence of food on longevity has received much attention in recent years. Rau (1910) made observations concerning the duration of life in the saturniid moth, *Samia cecropia,* an insect that does not take food during its imaginal stage. Working with small samples, he reported that the mean length of life of the male was approximately 11 days and that of the female approximately 8 days. Rau and Rau (1912, 1914) reported that the mean length of life in various genera of the saturniid moth ranged from 6.53 days to 15.74 days. They found that the males of *Samia cecropia* (obtained from New York) lived longer than the females, that the males and females of *Tropaea luna* and *Samia cecropia* (obtained from St. Louis) showed no significant differences in mean duration of life, while the females of *Callosamia promethea* and *Telea polyphemus* lived longer than the males. Baumberger (1914) reported studies on longevity in insects without food. Many genera and species were used in this work. The imagoes were caught in a net and for this reason the true dates of hatching and consequently the true ages of the organisms at death were not known. Baumberger, however, concluded from his data that duration of life varies inversely with temperature and is not correlated with systematic groups. Kopec (1924) worked with *Lymantria dispar,* an insect which does not take food during the imaginal stage. He reported that intermittent starvation of the larvae produced a prolongation of the larval period but did not affect the mean duration of life of the adult. Pearl and Parker (1924) reported exact quantitative studies on the duration of life under condi-

tions of complete starvation of two varieties of *Drosophila melanogaster*. They found that under starvation the mean duration of life was almost the same in the vestigial as in the wild-type fly, although in a previous paper (Pearl and Parker, 1921) it was found that under conditions of full feeding the wild-type fly lived approximately three times as long as the vestigial. It was found that density of population had little effect on the length of life of the combined sexes under conditions of starvation. This finding is also in contrast to the results obtained when the flies were fed (Pearl and Parker, 1922). At all densities tested the females had a greater mean duration of life than the males. Lilliland (1938), working with *Drosophila pseudoobscura,* varied temperature, humidity and density of population. It was reported that without food the mean duration of life was greater at lower temperatures, lower densities and greater humidity. It was also reported that Race A lived longer than Race B. The differences were more pronounced at higher humidities.

Lutz (1915) used *Drosophila ampelophila* (*melanogaster*) in experiments dealing with duration of life. He allowed the flies to gain access to water but not to food. The results obtained when compared with more recent work (Pearl and Parker, 1924) showed that the addition of water increased the mean length of life. Loeb and Northrop (1916) performed the same experiment and reported that the mean duration of life varied inversely as the temperature between 9° C. and 34° C.

The reader is referred to Pearl (1928), Alpatov (1930), Pearl and Miner (1935) and Cowdry (1939) for more extensive bibliographies dealing with duration of life.

In the following investigation the duration of life of the wild-type fly of *Drosophila melanogaster* and its ebony mutant in the absence of food and water was studied. The wild-type fly was used as a control. The ebony mutant was used because, as was pointed out to the author by Dr. E. S. McDonough, its cultures thrived hardily under regular laboratory conditions.

The writer wishes to express his sincere appreciation to

Dr. E. S. McDonough, of the Department of Biology of Marquette University, for his constant help and encouragement throughout the course of this investigation. He wishes also to thank Dr. Raymond Pearl, of the Johns Hopkins University, for his helpful criticisms.

Materials and Methods

Experiments dealing with duration of life require that environmental factors be constant. A modified two-shelf incubator much like that of Bridges (1932) was constructed to maintain constant temperature. The thermoregulator and relay assembly was constructed according to Greiff (1939). The incubator during the course of the investigation ran constantly for five months with a temperature fluctuation of $\pm$ 0.05° C.

Pearl and Parker (1924) have pointed out the importance of controlling humidity so that there is no water present and at the same time no active desiccation of the flies. Accurate humidity control was accomplished by employing a saturated salt solution, as described by Obermiller (1924). Ammonium chloride (C.P.) was used. This solution has been reported to maintain a humidity of 79.3 per cent. at 25° C. (International Critical Tables, 1926). The solution was poured into a container and a mark made on the side of the container indicating the level of the solution. Care was taken to add only enough water to bring the solution level up to this mark. Crystals of ammonium chloride were added in excess to provide a margin of safety.

The stock cultures from which the flies used in this investigation were obtained were brought to Marquette University from Michigan State College in 1929. The flies were inbred for this investigation and the F_4 generation used. They were grown on a banana-agar medium and kept in the incubator at all times except as noted below.

The pupae comprising the F_4 generation were removed from the culture bottles by means of a flamed nichrome wire. The pupae were washed in 70 per cent. alcohol and

then put into individual test-tubes which had been previously plugged with cotton and sterilized. Each tube contained a strip of slightly moistened paper toweling upon which the pupa was placed. The tubes were placed in numbered racks and examined every 12 hours.

The technique used in handling the flies while they were in the incubator was that developed by Powsner (1935). Only four racks, each containing 30 test-tubes, were removed from the incubator at one time. It was found that no individual rack was out of the incubator more than 15 minutes in 24 hours.

DATA

Tables I and II give the survivorship distributions of

TABLE I

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA IN THE COMPLETE ABSENCE OF FOOD BASED ON 1,000 FLIES. SEXES COMBINED

Age (in hours)	Wild type	Ebony
6	1,000	1,000
12	998	1,000
24	997	998
36	956	997
48	562	932
60	121	899
72	14	319
84	4	78
96	0	13
108		2
120		1
132		0
Absolute no. of flies	766	784

TABLE II

SURVIVORSHIP DISTRIBUTIONS OF DROSOPHILA IN THE COMPLETE ABSENCE OF FOOD BASED ON 1,000 FLIES. SEXES SEPARATE

Age (in hours)	Wild type		Ebony	
	Male	Female	Male	Female
6	1,000	1,000	1,000	1,000
12	997	1,000	1,000	1,000
24	997	998	980	1,000
36	973	940	980	997
48	646	487	930	936
60	151	98	723	702
72	16	17	313	326
84	0	7	77	79
96		0	14	11
108			2	3
120			0	3
132				0
Absolute no. of flies	370	396	441	343

the wild-type fly and its ebony mutant. The tables were

calculated on the basis of 1,000 flies and were corrected to the nearest whole number. The survivorship lines are compared graphically in Figs. 1 to 3. Figs. 4 and 5 show

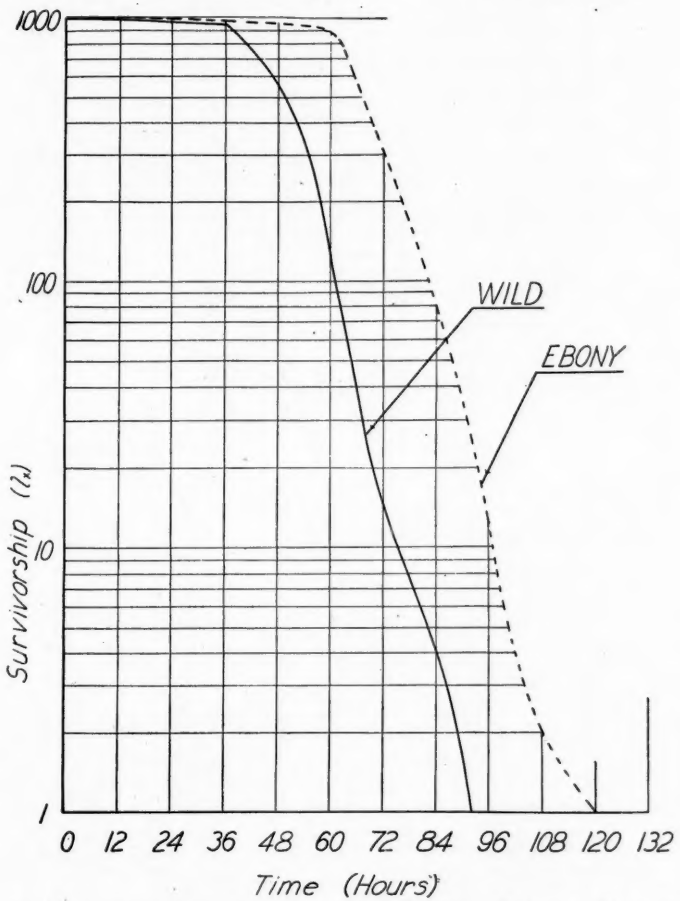

FIG. 1. A Comparison of the Wild Type and Ebony Mutant l_x Lines of *Drosophila.*

the death curves (d_x) and the survivorship curves (l_x) placed on a relative time base for the purpose of comparison (Pearl, 1927).

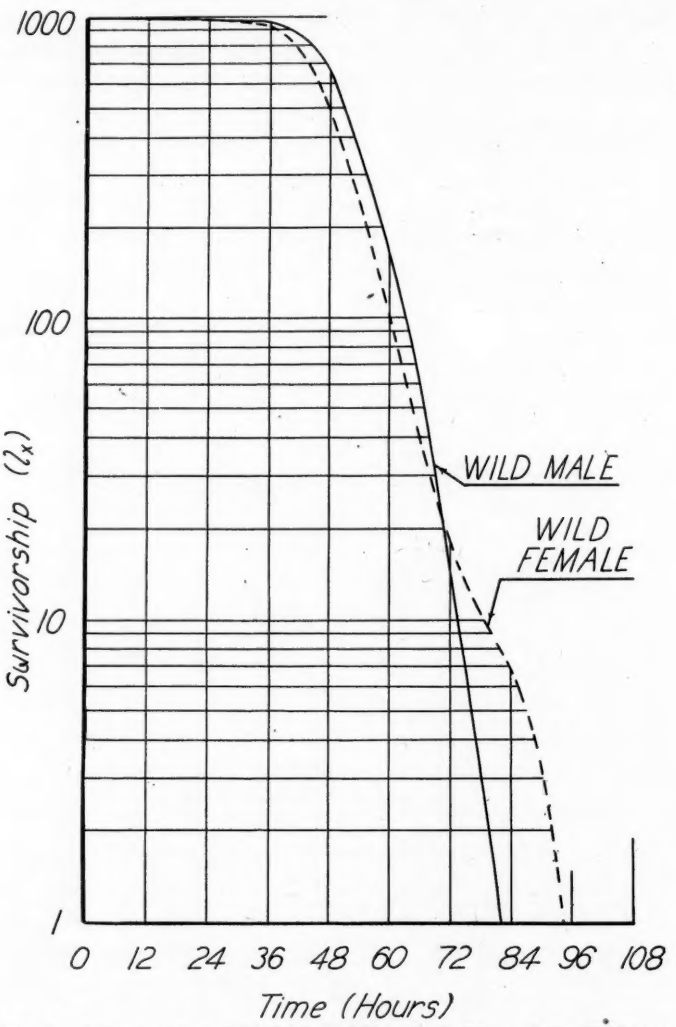

FIG. 2. A Comparison of the Male and Female 1_x Lines of the Wild-type *Drosophila*.

The chief biometric constants for this investigation are given in Table III.

The mean duration of life of the ebony fly, sexes com-

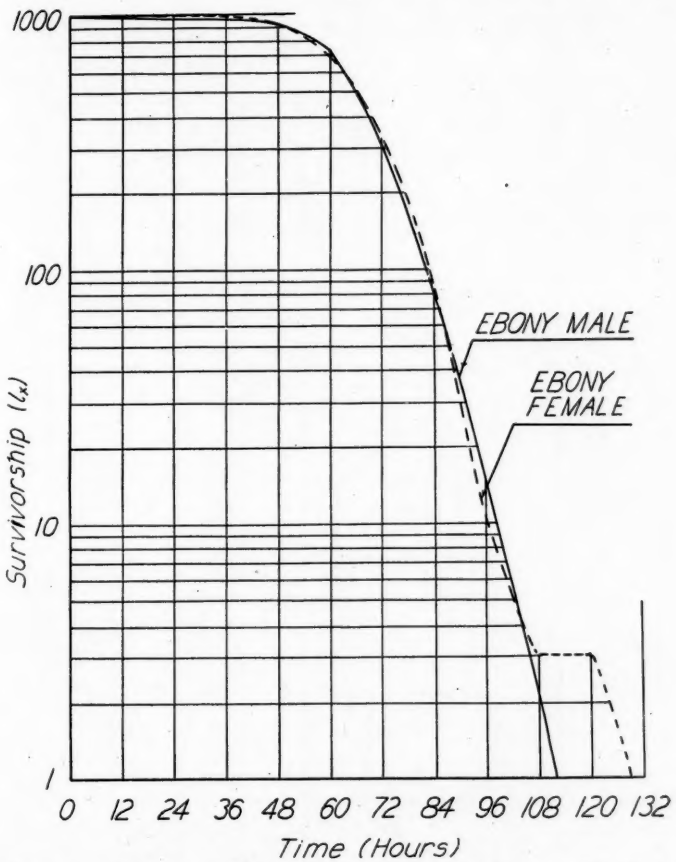

FIG. 3. A Comparison of the Male and Female 1_x Lines of the Ebony Mutant of *Drosophila*.

TABLE III

	Mean duration of life (hours)	Standard deviation (hours)	Standard deviation of mean (hours)
Wild-type fly (sexes combined)	49.89	9.77	.353
Ebony fly (sexes combined)	66.76	11.77	.420
Wild-type male	51.51	9.46	.492
Wild-type female	48.91	10.08	.507
Ebony male	66.80	12.82	.609
Ebony female	66.89	12.99	.702

bined, was found to exceed that of the wild-type fly, sexes combined, by 16.77 hours. The standard error of the

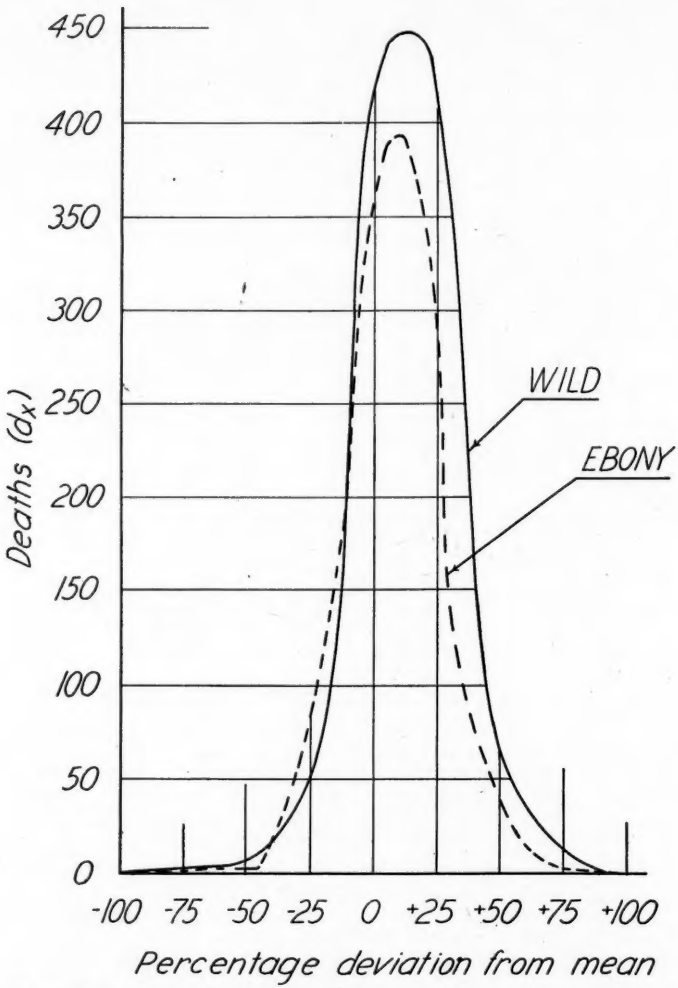

FIG. 4. Death Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

difference being .55 the difference was significant. In the foregoing and the following determinations of significance

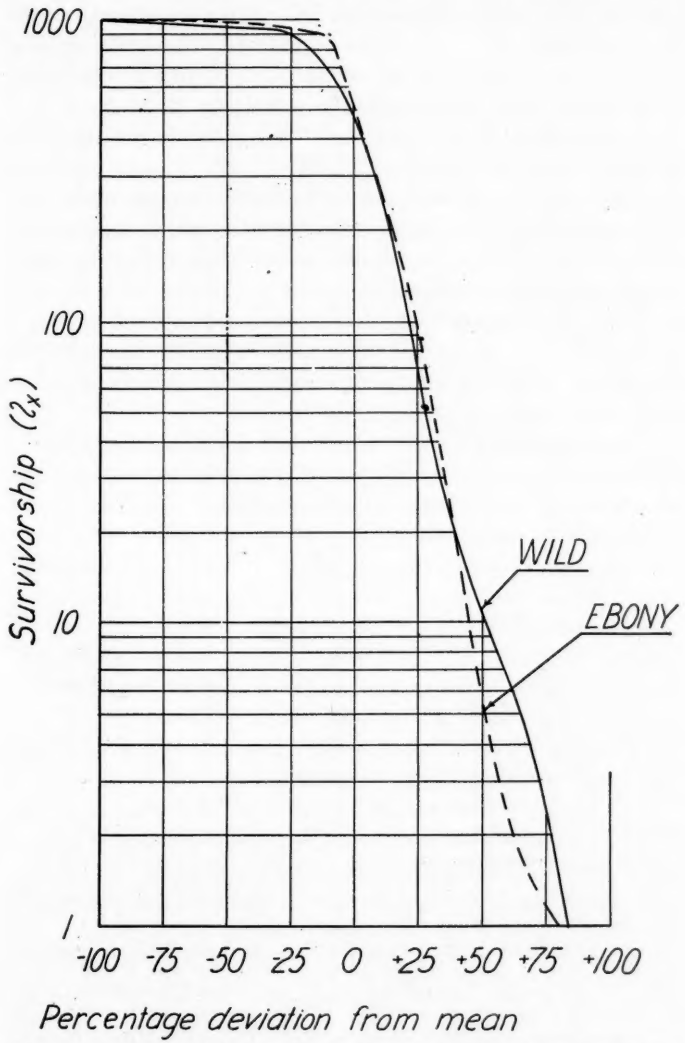

FIG. 5. Survivorship Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

or non-significance "Student's" *t*-test for unique examples was used.

The mean duration of life of the ebony female was found to exceed that of the ebony male by .09 hours. The standard error of the difference being .92 hours, the difference was not significant.

The mean duration of life of the wild-type male was found to exceed that of the wild-type female by 2.60 hours. The standard error of the difference being .707 hours, the difference was significant.

The mean duration of life of the ebony male was found to exceed that of the wild-type male by 15.29 hours. The standard error of the difference being .805 hours, the difference was significant.

The mean duration of life of the ebony female was found to exceed that of the wild-type female by 17.98 hours. The standard error of the difference being .85 hours, the difference was significant.

DISCUSSION

In this investigation the mean length of life of the ebony mutant fly under starvation was found to be significantly greater than that of the wild-type fly under starvation. The mutant ebony gene apparently affects not only the coloration of the fly but also the summation of the physiological processes so that the mean length of life increases under the conditions of the investigation. Jennings (1939), in speaking of the work on longevity performed by Gonzalez (1923), says:

> As is well known, every gene (with seemingly few exceptions) plays an essential rôle in the life and development of every cell of the body. It is not surprising therefore that changing a single gene may so alter the cellular processes as to change the length of life.

Two explanations for the difference in mean length of life under starvation are possible. Using the duration of life of the imago as an index of its "rate of living" (Pearl, 1928) we may say that the "rate of living" was slower in the ebony mutant than in the wild-type fly under conditions of starvation. It is also possible that the ebony mutant

imago possesses a greater "capital" than the wild-type fly and maintains its advantage throughout life (Ashby, 1930, 1932).

Flies grown under laboratory conditions, where often there are in excess of 200 flies to a bottle, may be considered as under a condition of partial starvation. It may be reasonably stated that those flies which lived longer under a condition of complete starvation would retain this advantage under a condition of partial starvation. As was pointed out in the beginning of this report, the ebony mutant fly was observed to do better under laboratory conditions than the wild-type fly.

Pearl and Parker (1924) in reporting their work on the wild-type fly and its vestigial mutant under starvation used three different densities of population, these being fly densities of 5, 50 and 100 per container. They reported that the mean duration of life of the vestigial mutant under these conditions was much the same as the mean duration of life of the wild-type fly. Pearl and Parker, however, allowed males and females to live together for a short time. Krumbiegel (1929) reported that cohabitation of males and females shortened the mean duration of life in both sexes of Drosophila. The mean duration of life of the vestigial and wild-type fly under a density of one might give dissimilar life curves. On the other hand, it is not improbable that the effects of living together vary in different varieties of flies.

The survivorship curves for the ebony mutant fly and the wild-type fly, when placed on a relative time base, were found to be almost identical in shape (*cf.* Fig. 5), although their time placement when computed with regard to actual time is different. Pearl and Parker (1924) found that the form of the life curve under starvation was the same for the fed wild-type and starved wild-type and starved vestigial males. The life curve for the fed vestigial males, however, approached a straight diagonal on an arithlog grid.

The death curves for the ebony mutant and the wild-type fly were skewed to the left (*cf.* Fig. 4). The two

limbs of the curves were very close together and showed a small degree of variation. This gave rather sharply peaked, unimodal frequency curves. Pearl and Miner (1935) state that this type of curve can not be caused by accident. It has been suggested by them that one of the causes for this type of curve is a lethal agent of environmental origin. This lethal agent must be so powerful that it transcends individual variations attempting to nullify its power. Starvation in the present investigation seems to take the place of an externally administered poison. As soon as the endogenous source of energy is lessened to an appreciable extent the toxic substances of the organism accumulate and, after the threshold of toleration is passed, death results. Schlutz, Hastings and Morse (1933) working with mammals reported that inanition may cause the physiological machine to be less efficient for delivering oxygen and removing metabolic products from the tissue.

A study of the survivorship curves of the ebony mutant and the wild-type fly (*cf.* Figs. 2 and 3) brings out a very interesting fact. The last surviving individuals were female flies. Numerous experiments on both man and rats (Bodansky, 1934) have shown that basal metabolism is lowered by starvation. Orr (1937) reported that starvation reduces oxygen consumption in both sexes of Drosophila. An explanation of the above observation may be that the basal metabolism of the female decreases more rapidly than that of the male. Thus the "rate of living" of the female would become progressively lessened and as a consequence the length of life increased.

Many workers in the field of longevity have reported that the female is longer lived than the male. Thus Pearl and Parker (1924) state:

> The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.

It will be recalled that Pearl and Parker worked with flies at densities of 5, 50 and 100 per container. In the

author's investigation, in which the density was 1 per container, it was found that the wild-type male lived significantly longer than the wild-type female. In the work of Pearl and Parker, although the difference in the mean duration of life between male and female favored the female, this difference became progressively less as the density of population decreased. Plotting the differences of the means against the log of the density results in an approximately straight line. This line shows that there is a crossing over in the regions of low population densities and the mean duration of life of the male becomes greater than that of the female. Further experiments are being planned to check the foregoing observations.

Summary

This paper presented the results of the determination of duration of life in 1,550 adult individuals of *Drosophila melanogaster* and its ebony mutant, under a population density of one and conditions of starvation without water. The results obtained were:

(1) The mean duration of life of the ebony mutant was found to exceed in a statistically significant manner the mean length of life of the wild-type fly.

(2) The wild-type male was found, on the average, to live longer than the wild-type female. The difference was shown to be statistically significant.

(3) The mean duration of life of the ebony female was found to be greater than that of the ebony male. The difference was shown to be statistically non-significant.

(4) The last surviving individuals in both the ebony and wild-type populations were females.

(5) The life curves of the ebony and wild-type flies were found to have the same shape when placed on a relative time base.

LITERATURE CITED

Alpatov, W. W.
1930. Am. Nat., 64: 37–55.
Ashby, Eric
1930. *Annals of Botany*, 44: 457–467.
1932. *Annals of Botany*, 46: 1007–1032.

Baumberger, J. P.
1914. *Ann. Ent. Soc. Amer.*, 7: 323–353.
Bodansky, Meyer
1934. "Introduction to Physiological Chemistry." New York: John Wiley and Sons, Inc. Pp. xi + 662.
Bridges, C. B.
1932. AM. NAT., 66: 250–273.
Cowdry, E. V. (ed.)
1939. "Problems of Aging." Baltimore: Williams and Wilkins Company.
Gonzalez, B. M.
1923. AM. NAT., 57: 289–326.
Greiff, Donald
1939. *Science*, 89: 468.
Jennings, H. S.
1939. "Senescence and Death in Protozoa and Invertebrates," "Problems in Aging." Ed. by E. V. Cowdry.
Kopec, S.
1924. *Biol. Bull.*, 46: 1–21.
Krumbiegel, Ingo
1929. *Zool. Jahrb. Abt. Anat. u. Ontog. Tiere*, 51: 111–162.
Lilleland, Ole
1938. *Biol. Bull.*, 74: 314–318.
Loeb, J. and J. H. Northrop
1916. *Proc. Nat. Acad. Sci.*, 2: 456–457.
Lutz, F. E.
1915. *Bull. Amer. Mus. Nat. Hist.*, 34: 605–624.
Obermiller, J.
1924. *Zeitschr. f. Physik. Chem.*, 109: 145.
Orr, P. R.
1937. *Physiol. Zool.*, 10: 235–243.
Pearl, Raymond
1927. *Science*, 65: 237–241.
1928. "The Rate of Living." Pp. vii + 185. New York: Alfred Knopf.
Pearl, R. and J. R. Miner
1935. *Quart. Rev. Biol.*, 10: 60–79.
Pearl, R. and S. L. Parker
1921. AM. NAT., 55: 481–509.
1922. AM. NAT., 56: 312–322.
1929. AM. NAT., 63: 193–218.
Powsner, L.
1935. *Physiol. Zool.*, 8: 474–520.
Rau, P.
1910. *Trans. Acad. Sci. St. Louis*, 19: 21–48.
Rau, P. and N. Rau
1912. *Jour. Exper. Zool.*, 12: 179–204.
1914. *Trans. Acad. Sci. St. Louis*, 23: 1–78.
Schlutz, F. W., A. B. Hastings and M. Morse
1933. *Amer. Jour. Physiol.*, 104: 669–675.

SHORTER ARTICLES AND DISCUSSION

SEX-LINKAGE IN PTEROMALUS

DOZORCEVA (1936) has published data from her work with the Chalcidoid wasp *Pteromalus puparum* Linnaeus which are of much interest, as they extend to another superfamily of the Hymenoptera some of the principles of sex-determination demonstrated for the Ichneumonoid Habrobracon.

In the latter sex-determination is complementary and a series of multiple allelic sex factors is postulated. Females are heterozygous for sex (*xa/xb, xa/xc,* etc.) and when unmated produce haploid sons of two types in equal numbers (*xa* and *xb, xa* and *xc,* etc.). When females are mated about two thirds of their eggs are fertilized. If parents are "unrelated," having different alleles ($xa/xb \times xc$), all zygotes are females (*xa/xc* and *xb/xc*): if "related" ($xa/xb \times xa$ or $xa/xc \times xc$), one half the zygotes are potentially diploid males (*xa/xa, xc/xc*). These are highly inviable. Fused, *fu,* is a sex-linked recessive about ten crossover units from the sex-differentiating factor. In close-crosses of heterozygous female (*xa/xb.fu*) by fused male (*xa.fu*) sex-linkage is apparent, since all four types of zygotes (straights and crossovers) are separable, but in outcrosses ($xa/xb.fu \times xc.fu$) linkage can not be detected, since all zygotes are females.

In Pteromalus, red-eye, *r,* is recessive to wild-type black. Sixty-nine unmated heterozygous females produced males—+ 3,224, *r* 2,875, giving a relative viability ratio of 0.89 + for red. Mated wild-type by wild-type produced 2,878 females, 3,090 males; mated red by red 102 females, 187 males. Female ratio is therefore 0.475.

Data are not given for crosses of red females by wild-type males, and thus no diploid (biparental) males are demonstrated. However, the similarity of the female ratio, 0.475, to that of inbred lines of Habrobracon suggests that many of the crosses may be of "related" parents (close-crosses) with half the fertilized eggs inviable or producing a few diploid males. In outcrosses of Habrobracon, female ratio is 0.67 or higher, and there are no diploid males.

Dozorceva obtained progeny from ten matings of heterozygous Pteromalus females by red males. Normal sons, 433, exceeded red, 357, because of viability difference as among progeny of

unmated mothers. The ten fraternities fell into three groups as regards eye color of females. One showed near equality—+39, *r* 43. One showed marked excess of red—+13, *r* 33, while each of the other eight had no red or very few totalling +270, *r* 22. These three types of fraternities are comparable in respect to red with fraternities in Habrobracon involving the sex-linked mutant-type fused. Equality of female types appears in outcrosses, disparity in close-crosses. Sex-linkage is shown by the latter only.

If the ten Pteromalus females be *xa.+/xb.r* (heterozygous for sex, *xa/xb*, as in Habrobracon), crosses to red males *xb.r* would give straights—wild-type females, *xa.+/xb.r*, and crossovers—red females, *xa.r/xb.r*. The diploid males (and inviable zygotes) resulting would be homozygous for sex (*xb.r/xb.r* and *xb.+/xb.r*), but these would here be non-separable from their red and wild-type haploid brothers. A cross with red male *xa.r* would give straights—red females, *xb.r/xa.r*, and crossovers—wild-type females, *xb.+/xa.r*. The male-producing zygotes homozygous for sex would be *xa.+/xa.r* and *xa.r/xa.r*. For eight close-cross fraternities in Pteromalus, the crossover females (red) were 22, the straights (+) 270. For one close-cross fraternity the crossovers (+) were 13, the straights (red) 33. Since the mutant-type has lowered viability, the true gametic ratio (recombinations to straights) may be obtained from the square root of the products $(\sqrt{22\times13}:\sqrt{270\times33})$ from which the crossover ratio is found to be 15.2.

Among the males ratios of red fluctuate near or somewhat below equality, but in one (probably a misprint as the total does not check) it is given as red 12, wild-type 65. The total is given as red 357, wild-type 433, a relative viability ratio for red (0.825) not far from that from unmated females (0.89+).

Sex ratio differs considerably in the different fraternities. In one (♀♀ 12, ♂♂ 266) the great excess of males is undoubtedly due to deficient sperm supply from the mating. The one fraternity giving near equality of the two colors in the females is comparable to outcross fraternities in Habrobracon, where sex-linkage is masked. This has the highest female ratio of all (♀♀ 82, ♂♂ 12) suggesting that all fertilized eggs were female-producing and therefore highly viable. Thus this "outcross" in Pteromalus may have been heterozygous female *xa.+/xb.r* by red male *xc.r*. The zygotes would all be female-producing because heterozygous

for sex (*xa/xc* and *xb/xc*) and hence wild-type and red would appear in equal numbers despite sex-linkage.

Greenshields (1939) has considered Dozorceva's data and has arrived at the conclusion "that it is impossible to derive any of the three categories of results if the genetics of sexuality and eye-color in *Pteromalus puparum* proceed according to the theory of P. W. Whiting." This conclusion is based upon a misunderstanding of my theory. According to Greenshields, half of the eggs are unfertilized by sperm from a given male and are, hence, male-producing; of the other half some are unfertilized and hence also male-producing. If this were true males would always exceed females, but this is contrary to fact not only for Pteromalus but for Habrobracon as well where females either equal males or exceed them. The misunderstanding probably traces back to my use of the terms selective fertilization, selective syngamy and differential maturation. These terms were used to indicate a selective process occurring *within* the egg *after* the sperm had entered and tending to increase the number of females. According to Greenshields' understanding, sperm would apparently be excluded from half of the eggs, thus tending to increase the number of males. The hypothesis of differential maturation, devised to explain high female-ratio in outcrosses, has now been given up in favor of the theory of multiple alleles.

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LITERATURE CITED

Dozorceva, R. L.
1936. *C. R. (Doklady) de l'Académie des Sciences de l'URSS*, III(XII), 7(102): 335–338.

Greenshields, F.
1939. AM. NAT., 73: 89–91.

THE OCCURRENCE OF DIPLOID MALES IN *HABROBRACON BREVICORNIS*

IN parasitic wasps of the genus Habrobracon males are normally produced by parthenogenesis and are therefore haploid. But the occurrence of exceptional diploid males from fertilized eggs has long been known in *H. juglandis* (Ashmead) (A. R. Whiting, 1927), and has recently been demonstrated in *H. pectinophorae* (Fumie Inaba, 1939). It is here reported in a third species of the same genus.

The standard genetic test for recognizing diploid males in *H. juglandis* is made by mating a female homozygous for a recessive gene to a male carrying the dominant allele. Normal haploid sons will show the matroclinous recessive trait, and diploid males can be distinguished by its absence since they develop from fertilized eggs and inherit the dominant allele from the father. Without such a genetic marker there is no certain way of recognizing diploid males except by cytological examination. Diploid cells are larger than haploid ones, and this increment is manifest in such mensurable characters as the number of bristles per unit area of wing surface and the size of eye facets, but these measurements vary so much with size variations among individuals that they are of more value statistically than for particular cases. The importance of the genetic marker to the recognition of diploid males is demonstrated by the fact that when P. W. Whiting reported the discovery of the first good mutant character found in *H. juglandis* he described this anomalous male type in the same paper (P. W. Whiting, 1921). It follows that diploid males may occur in many arrhenotokous species of bees and wasps where the genetics is unknown. Somewhat uncertain cases of patroclinous traits of males have been reported in the honey-bee, but diploid males have not been clearly demonstrated except in the genus Habrobracon.

In April, 1937, through the courtesy of A. B. Baird, a culture of *H. brevicornis* (Wesmael) was received from the Dominion Parasite Laboratory at Belleville, Ontario, where it is reared for use in combatting the European corn-borer, whose larvae are its normal host. It is being bred for comparison with *H. juglandis,* similar culture methods are employed and larvae of the Mediterranean flour-moth, *Ephestia kühniella,* which is the normal host of *H. juglandis,* are used for both. Morphologically, the two species are very similar, the chief points of difference being as follows. The antennae in both sexes are longer in *H. brevicornis;* the ground color of body and legs is redder; and the cocoons are longer, frequently are slightly curved, and fit the pupae very loosely.

The Whitings have previously bred *H. brevicornis* and descriptions of anomalous individuals of that species are included in several of their papers which deal chiefly with *H. juglandis* (P. W. Whiting and Anna R. Whiting, 1927; P. W. Whiting, 1932; P. W. Whiting, Raymond J. Greb and B. R. Speicher, 1934). Un-

fortunately, in early papers, before the identity of *H. juglandis* was correctly established, it was erroneously called *H. brevicornis* (P. W. Whiting, 1921). This has resulted in a confusion which still persists, as in a recent paper by Spotkov (Elias M. Spotkov, 1938), where he refers to Hase's work on *H. brevicornis* (A. Hase, 1922), while Hase's stock was actually *H. juglandis.*

Two mutations have been found in *H. brevicornis. Defective* causes the complete or partial disappearance of the fourth branch of the radius vein in one or both wings. It is a variable character and does not appear in all the sons of homozygous defective females. It does not reduce viability or fertility and closely resembles the mutation *defective* in *H. juglandis. Rough* is also very similar to the mutation of the same name in *H. juglandis.* Wing veins in the region of the fourth radial cell are not straight but irregularly formed with rough edges. In *H. brevicornis* the wings are sometimes extended out at the sides, though this feature does not appear in the majority of rough individuals. Homozygous females frequently produce no offspring, but this is probably due to general weakness rather than actual sterility, as they produce better when furnished host caterpillars previously stung by normal females.

In *H. juglandis* wild-type stocks are easily kept from generation to generation, the progeny of a mated female are allowed to eclose together, sibling matings occur and from these a high proportion of female offspring results. When this method is applied to *H. brevicornis,* however, the female ratio drops rapidly and the stock soon runs to males. This is partly due to the fact that *H. brevicornis* females resist mating, a condition which is now obviated by etherizing virgin females from stock and then placing them with males which readily mate with them.

However, even when females were so mated many of them produced only males. This led to the suspicion that diploid males may be produced in *H. brevicornis,* since in *H. juglandis* they are nearly sterile and females mated to them produce no daughters or very few. Genetic and cytological tests were accordingly made for diploid males. Sixteen rough females mated to type males produced 51 type females and 172 rough males, and in addition to these normal progeny produced 34 type males which were biparental, receiving the normal allele of the rough gene from the father. Cytological examination of these biparental males established the fact that they were diploid, since their

spermatocytes contained twenty chromosomes, while the normal haploid number is ten, as in *H. juglandis*.

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LITERATURE CITED

Hase, A.
1922. *Arb. a. d. biol. Reichanst. f. Land u. Forstwirtschaft*, 11: 95.
Inaba, Fumie
1939. *Cytologia*, 9: 517.
Spotkov, Elias M.
1938. AM. NAT., 72: 577.
Whiting, A. R.
1927. *Biol. Bull.*, 53: 438.
Whiting, P. W.
1921. *Biol. Bull.*, 41: 42.
Whiting, P. W.
1932. *Jour. of Comp. Psych.*, 14: 345.
Whiting, P. W., and Anna R. Whiting
1927. *Biol. Bull.*, 52: 89.
Whiting, P. W., Raymond J. Greb and B. R. Speicher
1934. *Biol. Bull.*, 66: 152.

BREAST RIDGE IN DOMESTIC FOWL, A NEW DOMINANT CHARACTER LINKED WITH PEA COMB, OR ANOTHER EXPRESSION OF THE PEA COMB GENE?

WHILE examining a group of dressed carcasses from a number of different breeds and crosses at the divisional killing plant in the summer of 1939, it was noted that all pure Cornish and first-cross Cornish carcasses possessed a peculiar ridgelike formation in the skin of the breast, running longitudinally in the median line just over the breast bone. This "breast ridge," as we have termed it, did not exist on the carcasses of pure-bred Barred Plymouth Rocks, White Leghorns or Light Sussex.

These observations suggested that the characteristic was inherited and led to the examination of living specimens. The findings on the live birds confirmed those made on the carcasses, and it was further found that the characteristic could be readily distinguished on young stock and even on baby chicks.

Fortunately, a number of Cornish crosses and testcrosses (backcrosses) were available and when all pertinent stock was examined it was found that "breast ridge" was present in all pure Cornish,

all F_1 individuals (Cornish × Leghorns and Cornish × Sussex) regardless of the direction in which the cross was made and in approximately 50 per cent. of the testcross progeny (Cornish-Leghorn × Leghorn). These data suggested that the character was a simple autosomal dominant, and additional testcross and F_2 matings were made to test this hypothesis.

In Table 1 are shown the data thus far obtained, compared with those expected on the basis of simple dominant autosomal inheritance.

TABLE 1

Type	Observed		Expected	
	Ridge	No ridge	Ridge	No ridge
F_1	75	0	75	0
F_2	34	14	36	12
Testcross	72	67	69.5	69.5

These data prove quite conclusively that the characteristic "breast ridge" is determined by a single dominant gene. Because the breast ridge is present in all F_1 individuals, regardless of the direction in which the cross was made, sex-linkage is effectively ruled out.

In the fall of 1939, the authors took advantage of the local poultry show to examine a large number of standard breeds and varieties for the presence of breast ridge. The characteristic was observed only in the Cornish, Cornish bantam, Black Sumatra and Brahma (light and dark) breeds of fowl. In other words, it was present only in pea-combed breeds.

The significance of the association between pea comb and breast ridge was not realized until it was noticed in the first F_2's obtained that all pea-combed birds had breast ridge and all single-combed individuals were smooth-breasted. A re-examination of the testcross data showed the same association and it immediately became apparent that breast ridge was either, (1) very closely linked with pea comb or (2) another expression of the pea-comb gene. Thereafter, careful observations were made on both breast ridge and pea comb in all F_2 and testcross individuals.

Of the 187 F_2 and testcross birds shown in the table presented in this report, all individuals with breast ridge possessed pea combs and all smooth-breasted birds had single-combs. However, in addition to these, there were three testcross individuals with pea combs which appear to be without breast ridges. In very

young chicks there is occasionally some difficulty in identifying breast ridge, and although one of these chicks is now six weeks old with no sign of a ridge, the association between pea comb and breast ridge has been so consistent, we hesitate to claim that we have secured any true cross-overs. It is possible that our three apparent cross-overs have very faintly expressed breast ridges due to the presence of genes modifying the expression of dominance in this characteristic.

We may conclude, then, that breast ridge is determined by a single autosomal dominant gene located in the same linkage group as pea comb, marbling and naked neck to which Bruckner and Hutt (1939) recently have added the blue-egg gene. Whether this gene is identical with that determining pea comb or very closely linked with it can not be definitely stated at the moment. Further data will be collected which will settle this point in the near future.

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LITERATURE CITED

Bruckner, J. H. and F. B. Hutt
1939. *Science*, 90: 88.